Figure 1

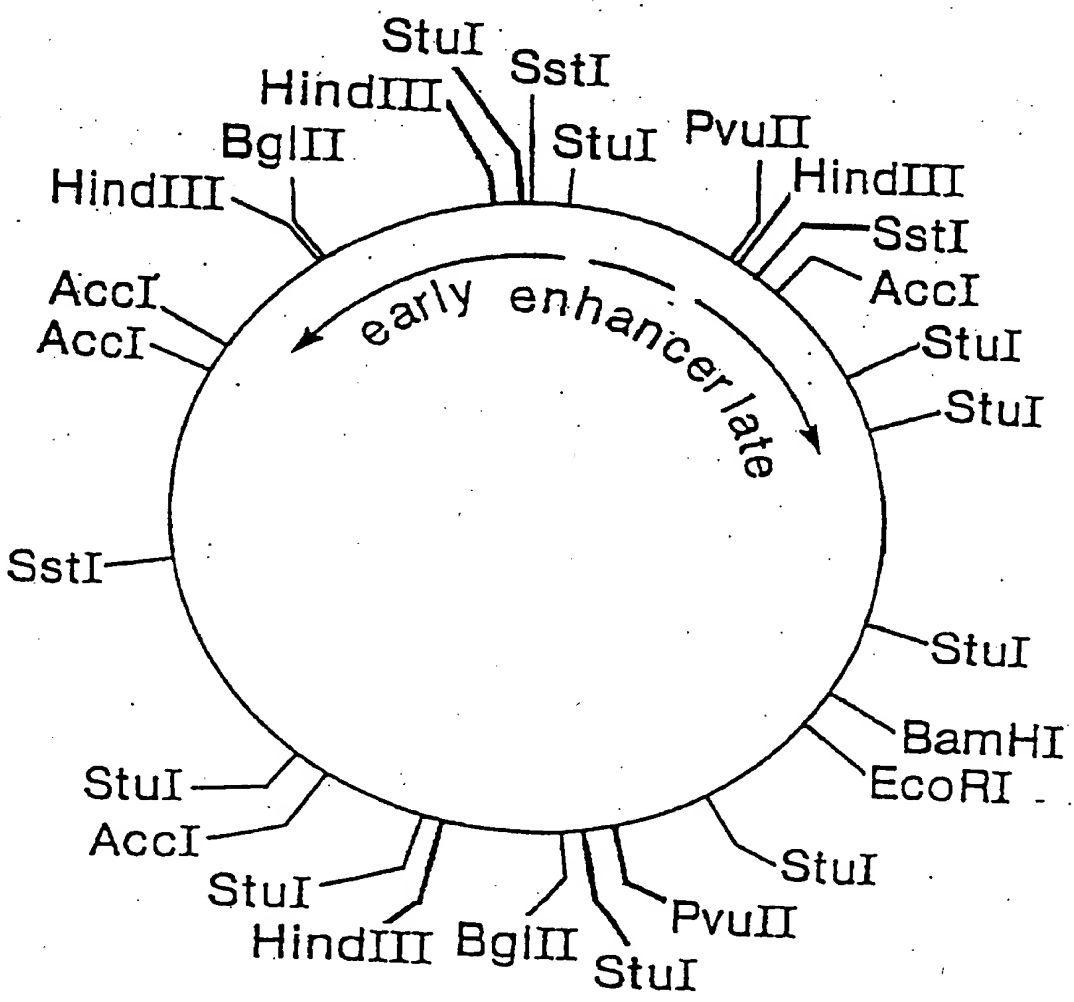

StuI
SstI
HindIII
BglII
StuI
PvuII
HindIII
HindIII
SstI
AccI
AccI
AccI
StuI
StuI
early enhancer late
SstI
StuI
BamHI
EcoRI
StuI
AccI
StuI
PvuII
HindIII
BglII
StuI

Figure 2

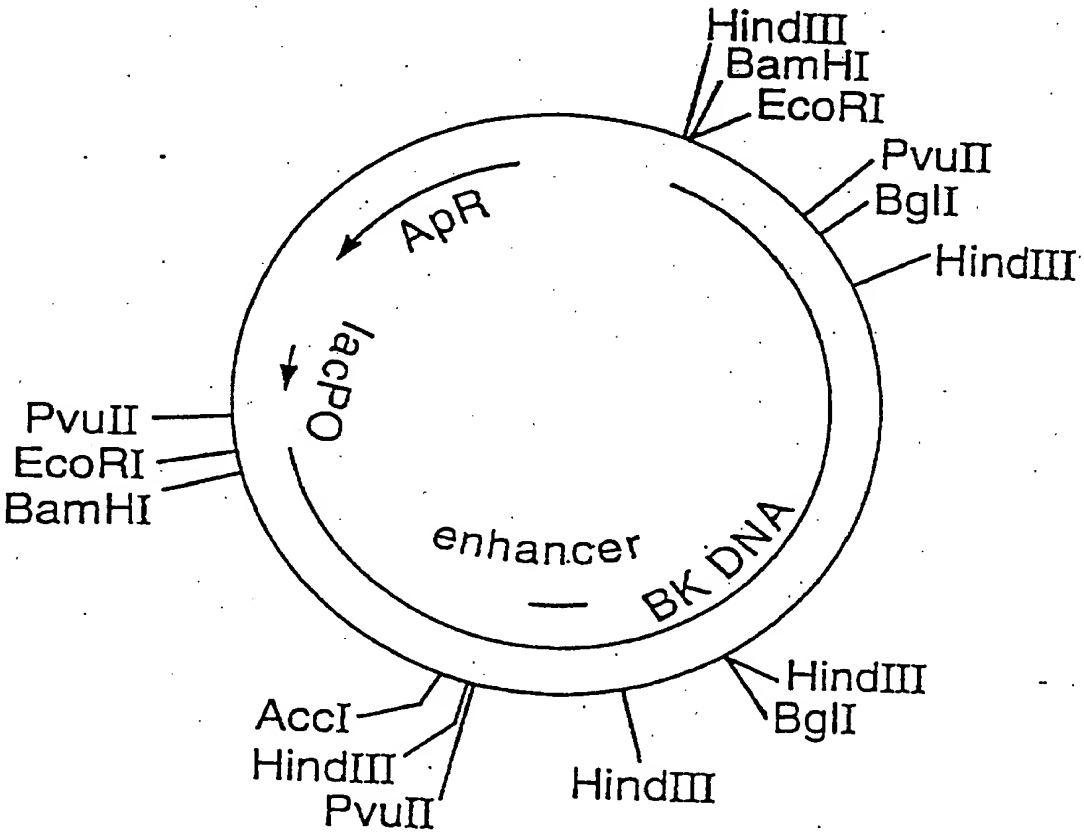

HindIII
BamHI
EcoRI
PvuII
BglI
HindIII
ApR
lacPO
PvuII
EcoRI
BamHI
enhancer
BK DNA
HindIII
BglI
AccI
HindIII
PvuII
HindIII

Figure 3

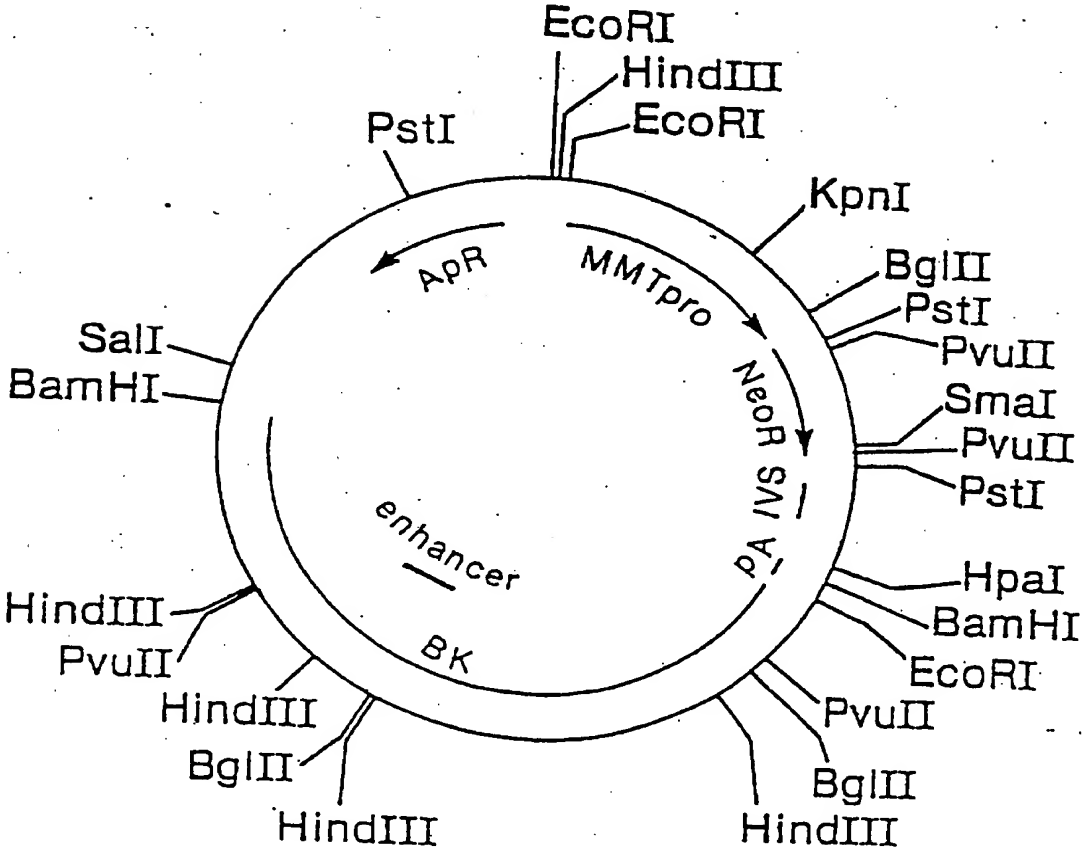

EcoRI
HindIII
EcoRI
PstI
KpnI
BglII
PstI
PvuII
SmaI
PvuII
PstI
HpaI
BamHI
EcoRI
PvuII
BglII
HindIII
SalI
BamHI
HindIII
PvuII
HindIII
BglII
HindIII
ApR
MMTpro
NeoR
SV A
IVS
pA
enhancer
BK

Figure 4

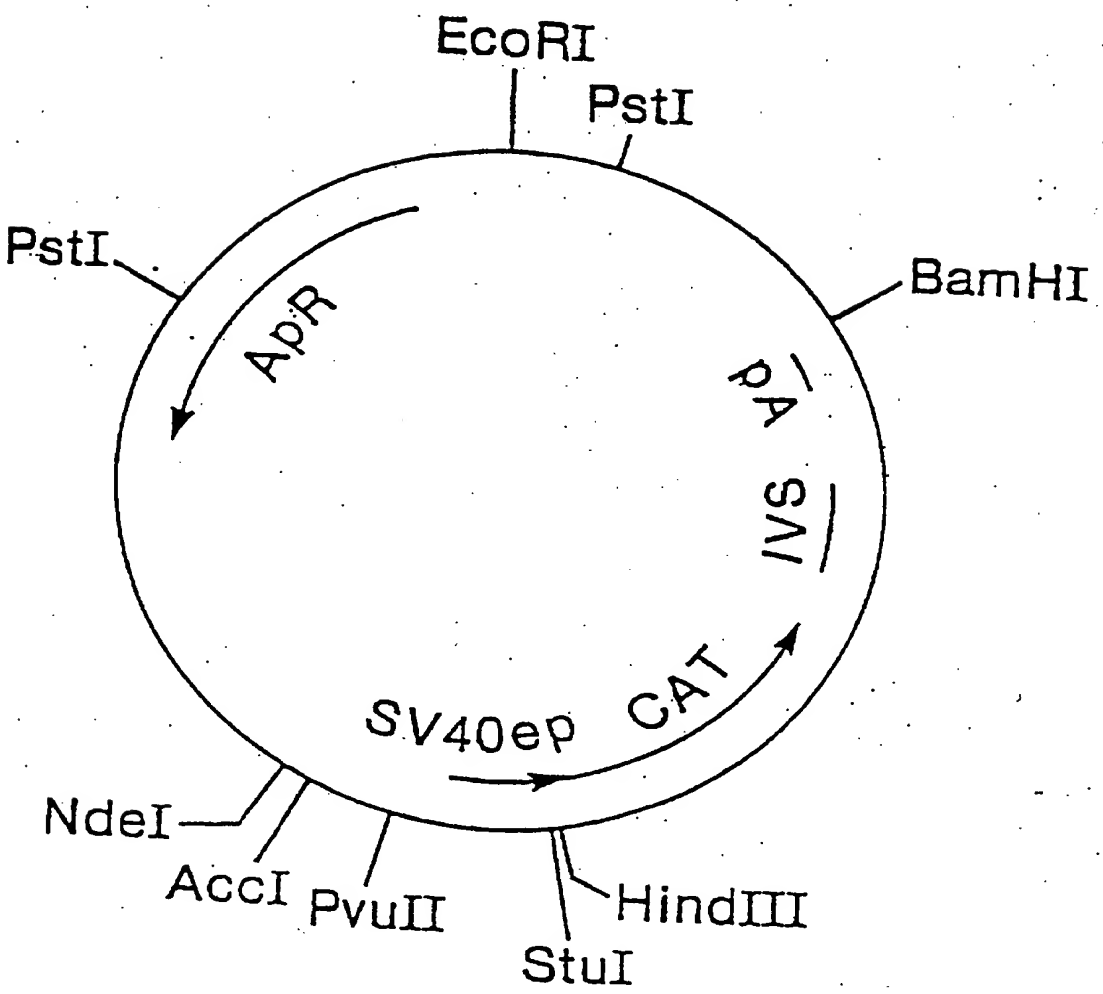

EcoRI
PstI
PstI
BamHI
ApR
pA
IVS
CAT
SV40ep
NdeI
AccI
PvuII
StuI
HindIII

Figure 5

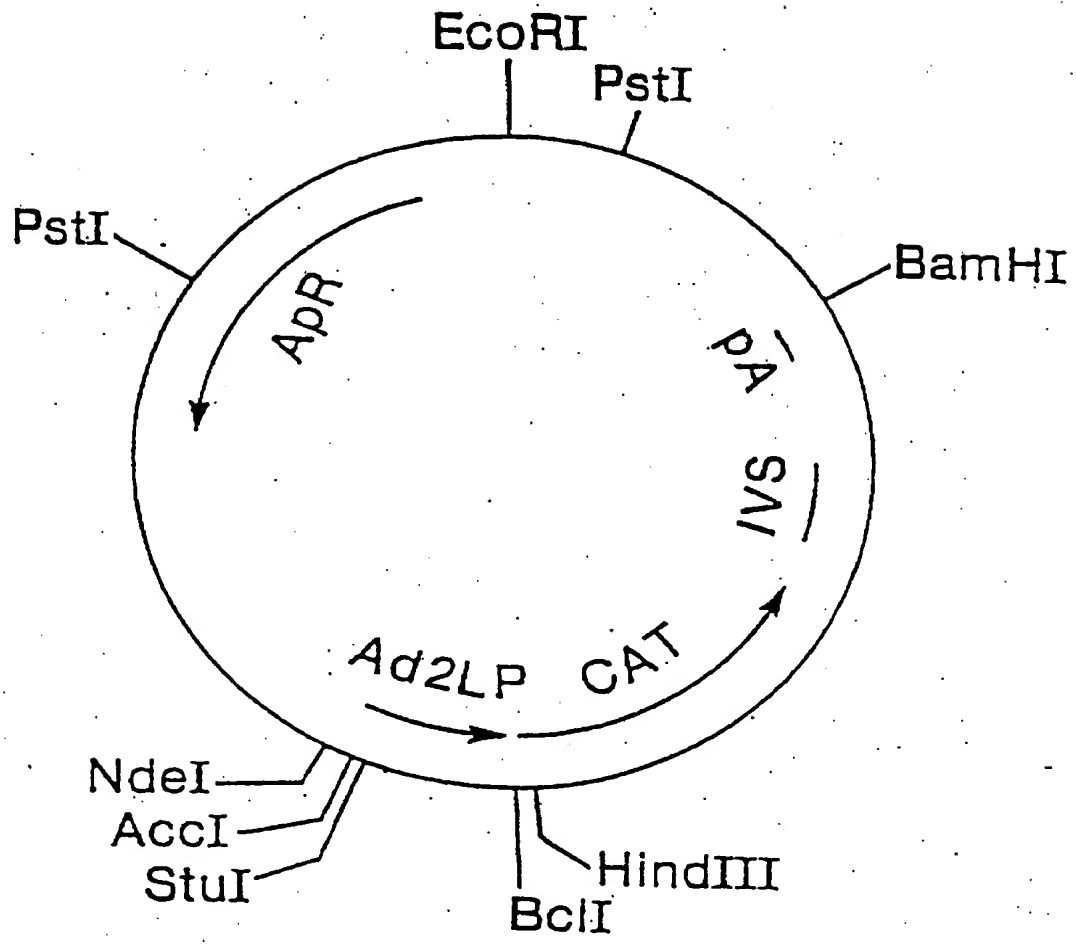

EcoRI
PstI
PstI
BamHI
ApR
pA
IVS
Ad2LP
CAT
NdeI
AccI
StuI
BclI
HindIII

Figure 6

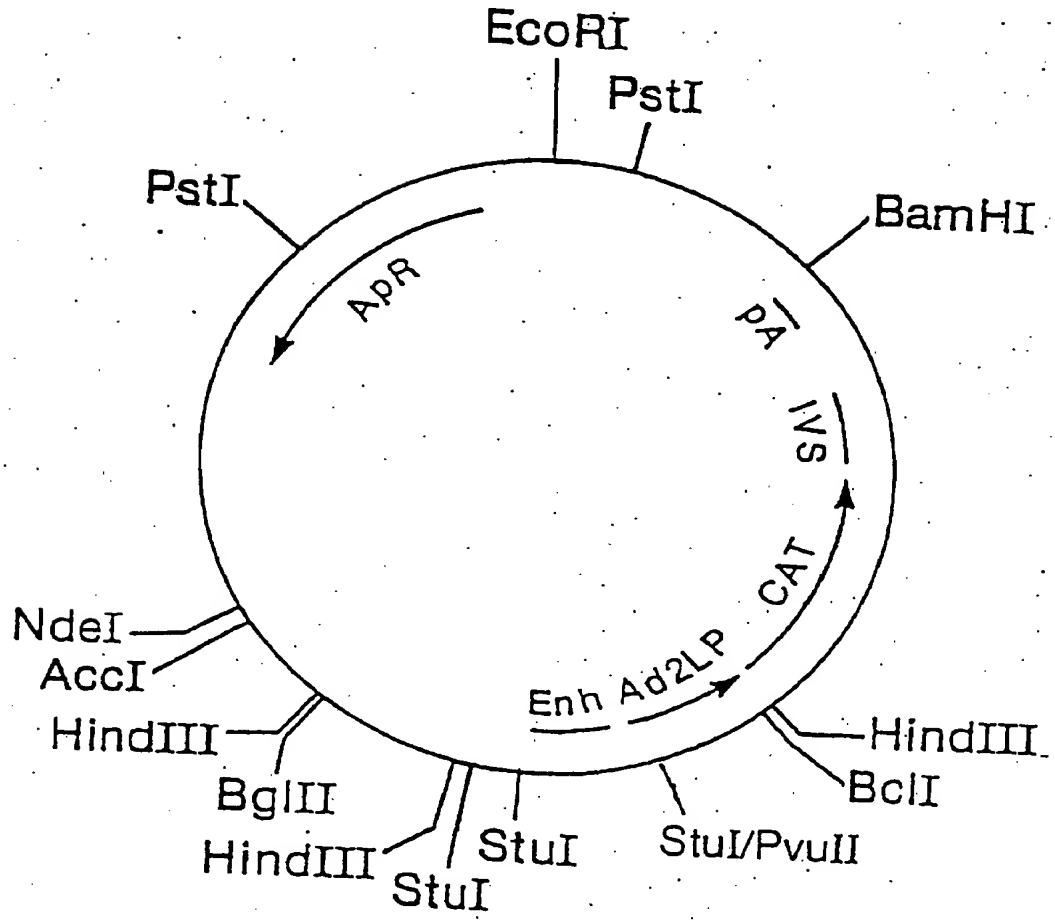

EcoRI
PstI
PstI
BamHI
ApR
pA
IVS
CAT
Enh Ad2LP
NdeI
AccI
HindIII
BglII
HindIII
StuI
StuI
StuI/PvuII
HindIII
BclI

Figure 7

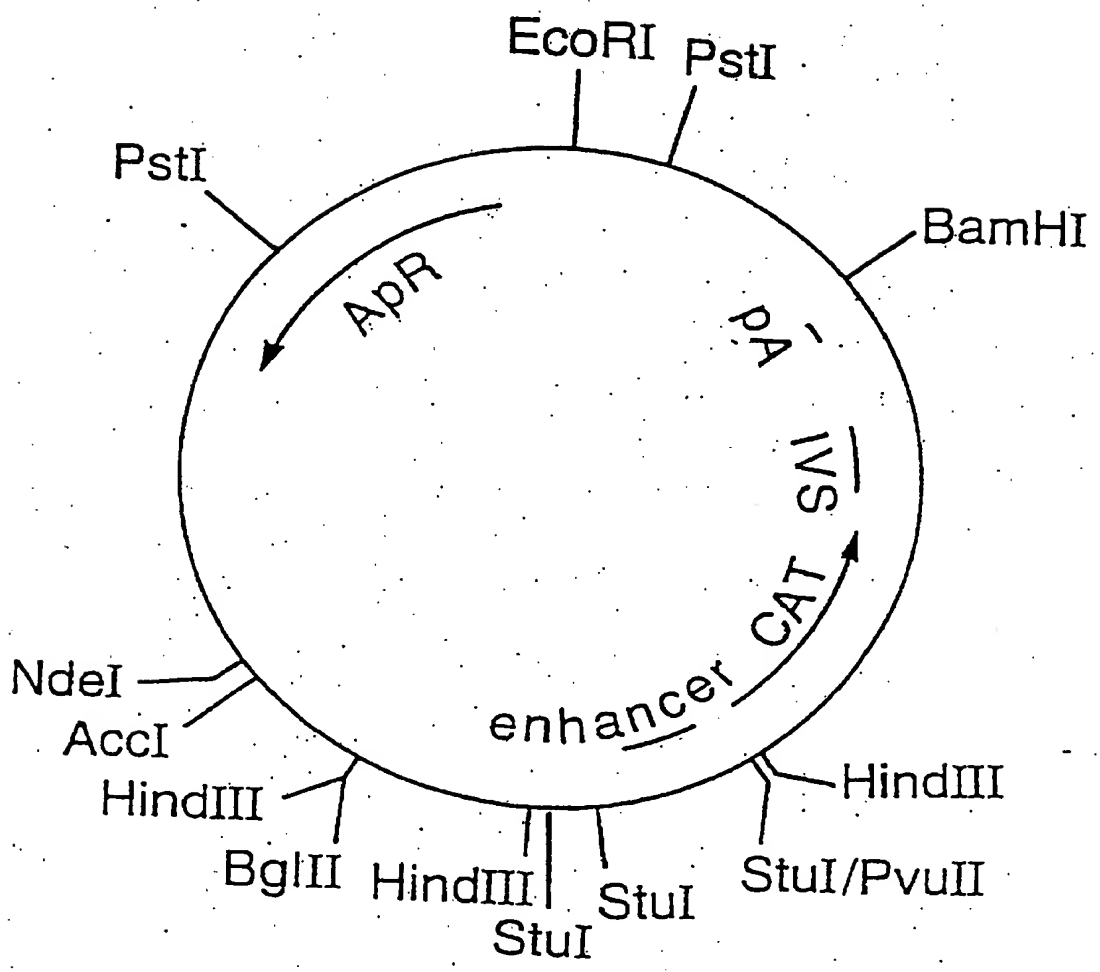

EcoRI
PstI
PstI
BamHI
ApR
pA
IVS
CAT
enhancer
NdeI
AccI
HindIII
BglII
HindIII
StuI
StuI
StuI/PvuII
HindIII

Figure 8

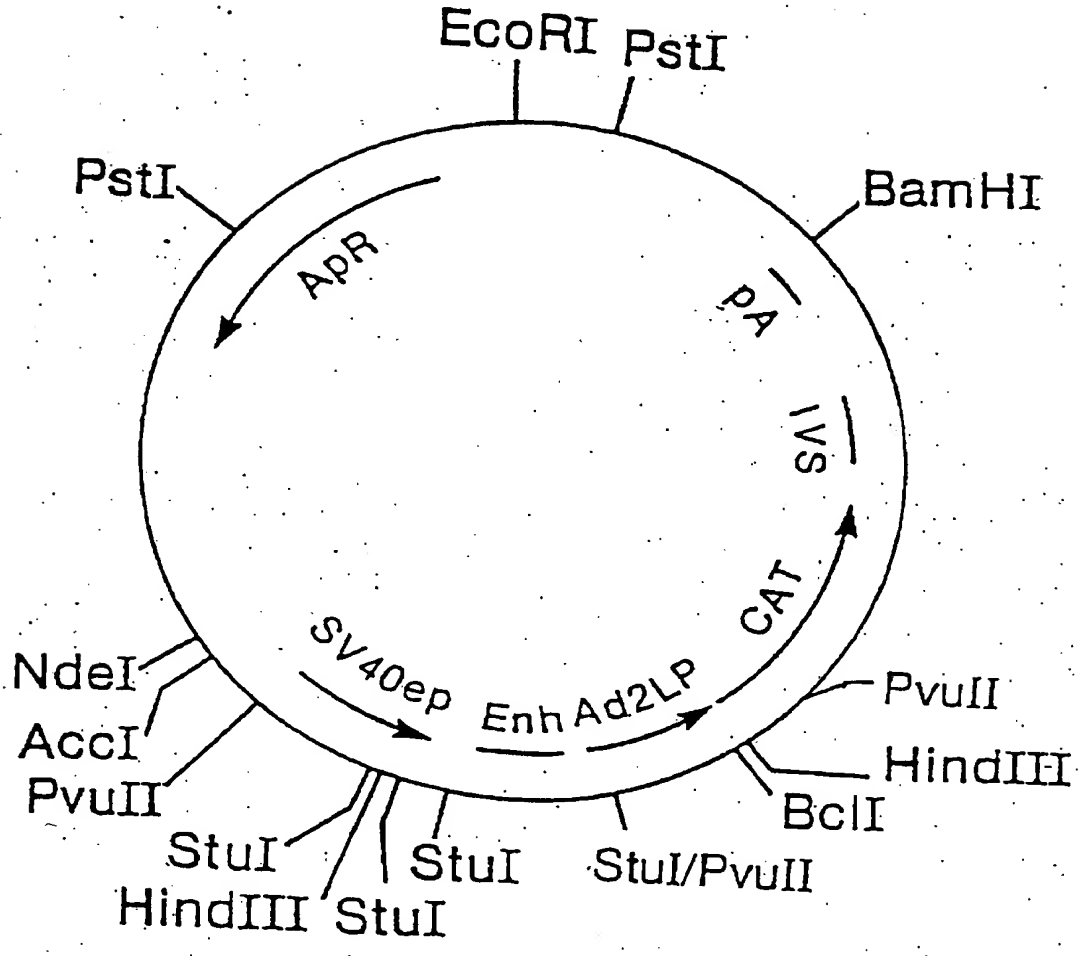

EcoRI
PstI
PstI
BamHI
ApR
pA
IVS
CAT
SV40ep
Enh Ad2LP
NdeI
AccI
PvuII
PvuII
HindIII
BclI
StuI
HindIII
StuI
StuI
StuI/PvuII

Figure 9 B

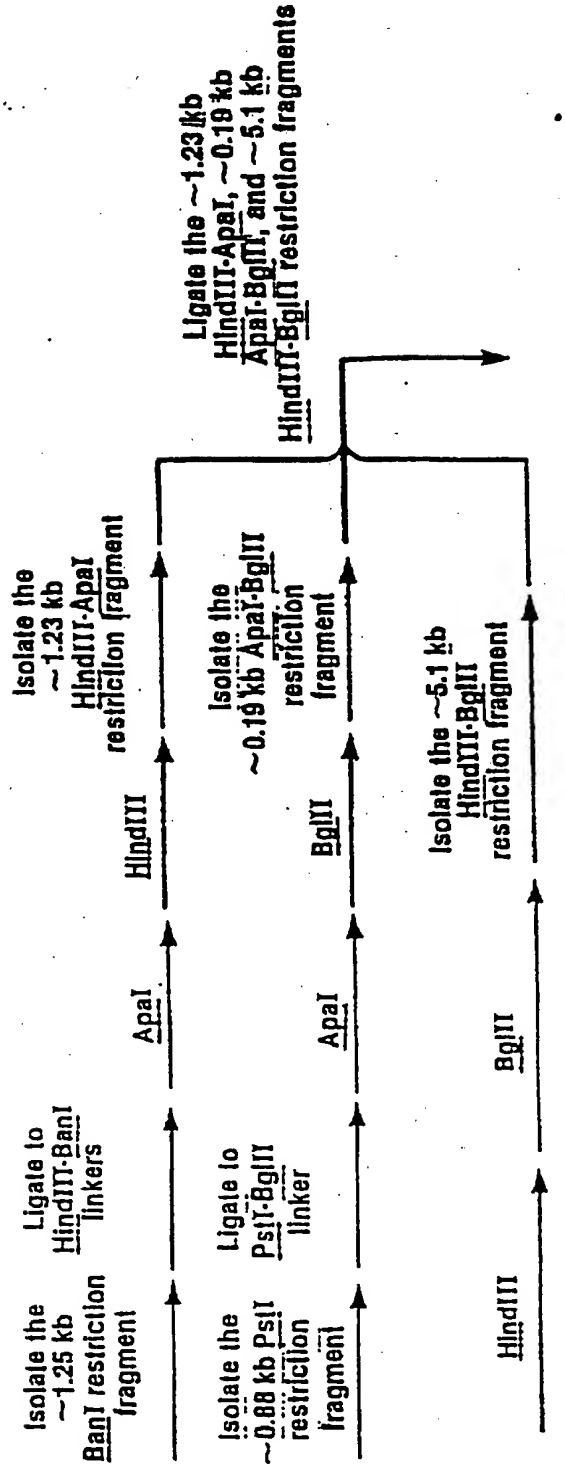

Isolate the ~1.25 kb BanI restriction fragment
Ligate to HindIII-BanI linkers
ApaI
HindIII
Isolate the ~1.23 kb HindIII-ApaI restriction fragment
Isolate the ~0.88 kb PstI restriction fragment
Ligate to PstI-BglII linker
ApaI
BglII
Isolate the ~0.19 kb ApaI-BglII restriction fragment
HindIII
BglII
Isolate the ~5.1 kb HindIII-BglII restriction fragment
Ligate the ~1.23 kb HindIII-ApaI, ~0.19 kb ApaI-BglII, and ~5.1 kb HindIII-BglII restriction fragments

Figure 9 C

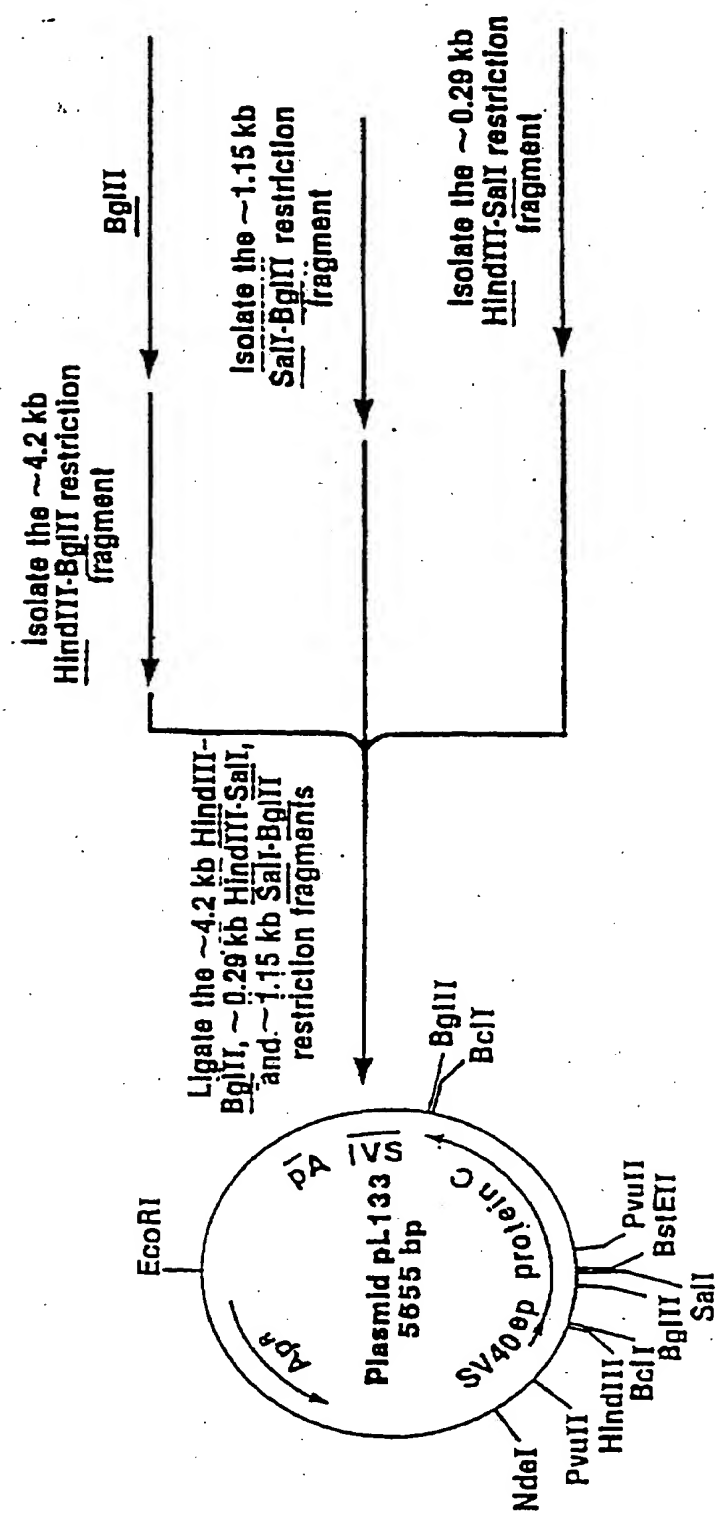

Isolate the ~4.2 kb HindIII-BglII restriction fragment
BglII
Isolate the ~1.15 kb SalI-BglII restriction fragment
Isolate the ~0.29 kb HindIII-SalI restriction fragment
Ligate the ~4.2 kb HindIII-BglII, ~0.29 kb HindIII-SalI, and ~1.15 kb SalI-BglII restriction fragments
BglII
BclI
pL
pA
IVS
C
Plasmid pL133
5655 bp
protein
SV40ep
EcoRI
PvuII
BstEII
SalI
BglII
BclI
HindIII
PvuII
NdeI
Ap^R

Figure 9 D

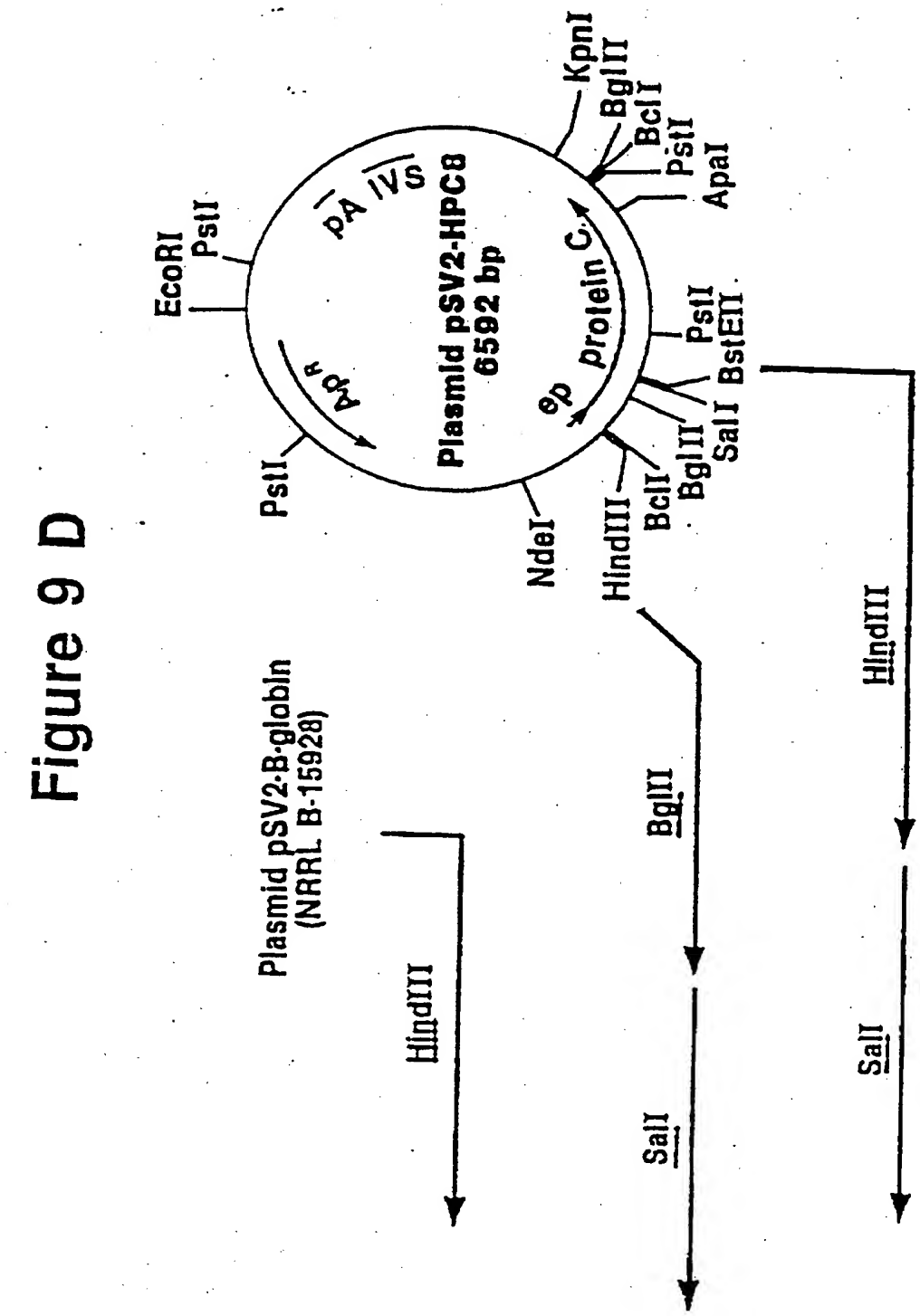

Plasmid pSV2-HPC8
6592 bp
KpnI
BglII
BclI
PstI
ApaI
PstII
BstEII
SalI
BglII
BclI
HindIII
NdeI
PstII
EcoRI
PstII
pA
IVS
Protein C
Plasmid pSV2-B-globin
(NRRL B-15928)
HindIII
BglII
SalI
HindIII
SalI

Figure 10

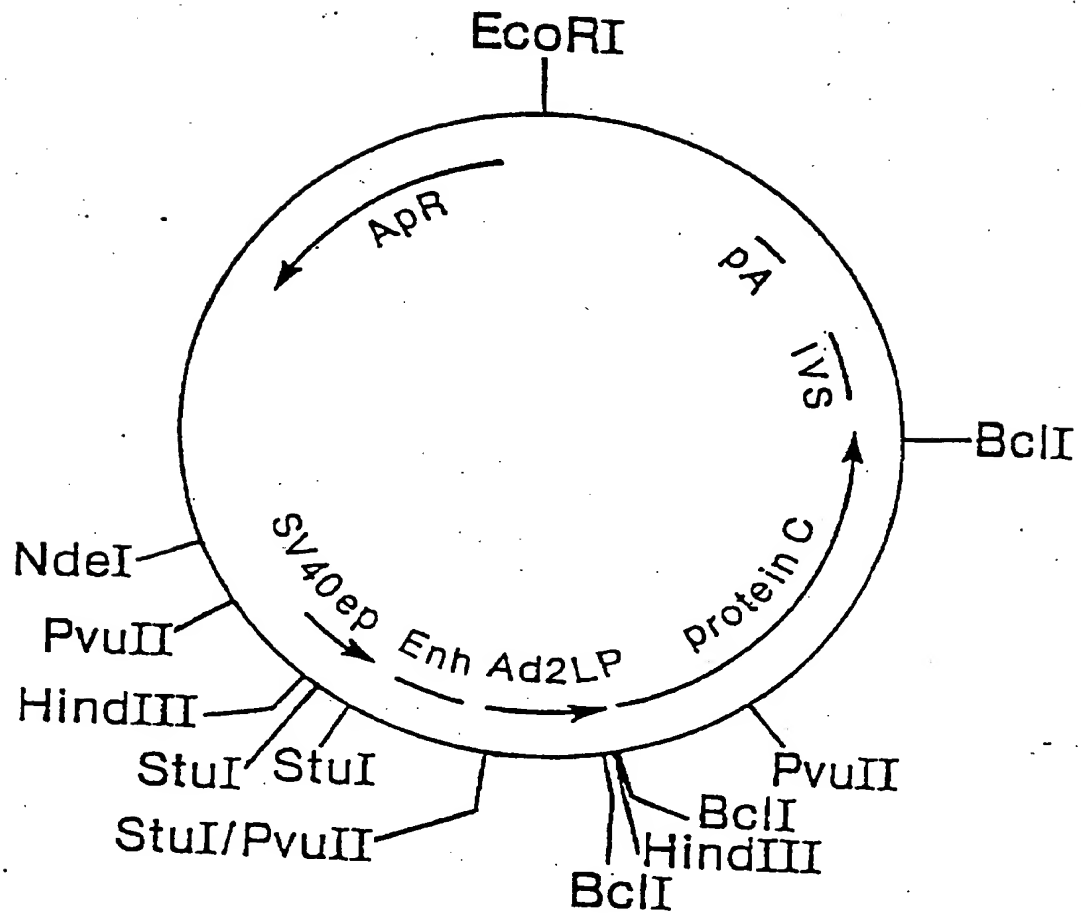

EcoRI
ApR
pA
IVS
BclI
SV40ep
Enh Ad2LP
protein C
NdeI
PvuII
HindIII
StuI
StuI
StuI/PvuII
PvuII
BclI
HindIII
BclI

Figure 11

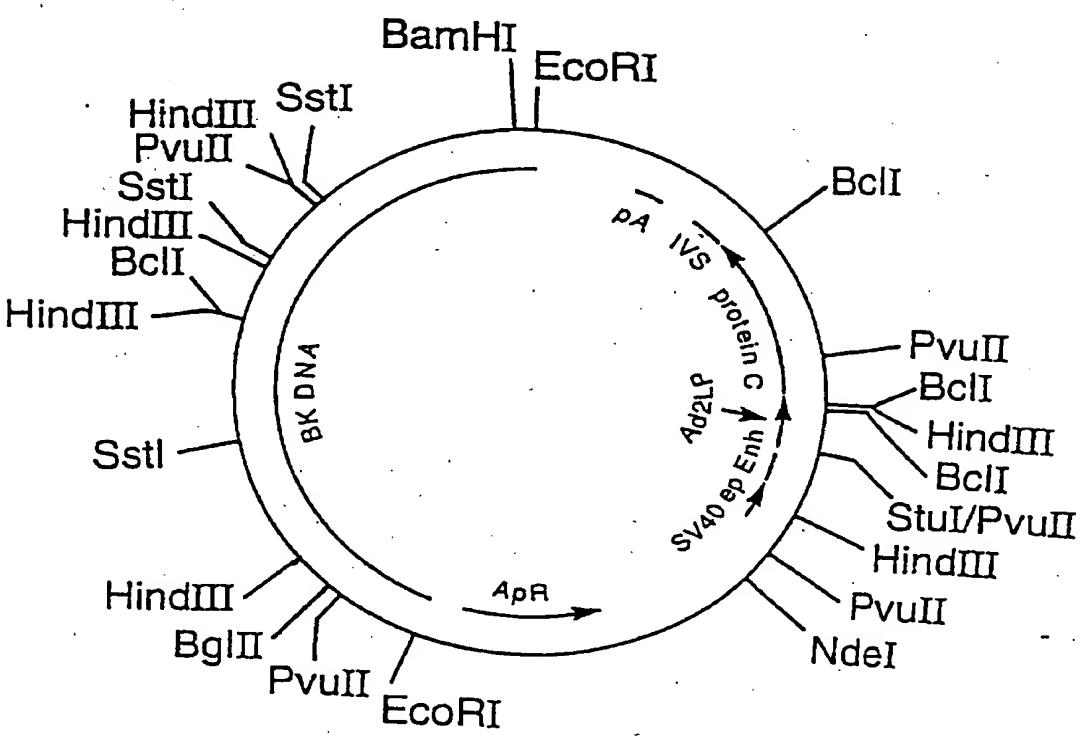

BamHI
EcoRI
BclI
PvuII
BclI
HindIII
StuI/PvuII
HindIII
PvuII
NdeI
EcoRI
PvuII
BglII
HindIII
SstI
HindIII
BclI
HindIII
SstI
HindIII
BK DNA
ApR
SV40 ep Enh
Ad2LP
protein C
IVS
pA

Figure 12

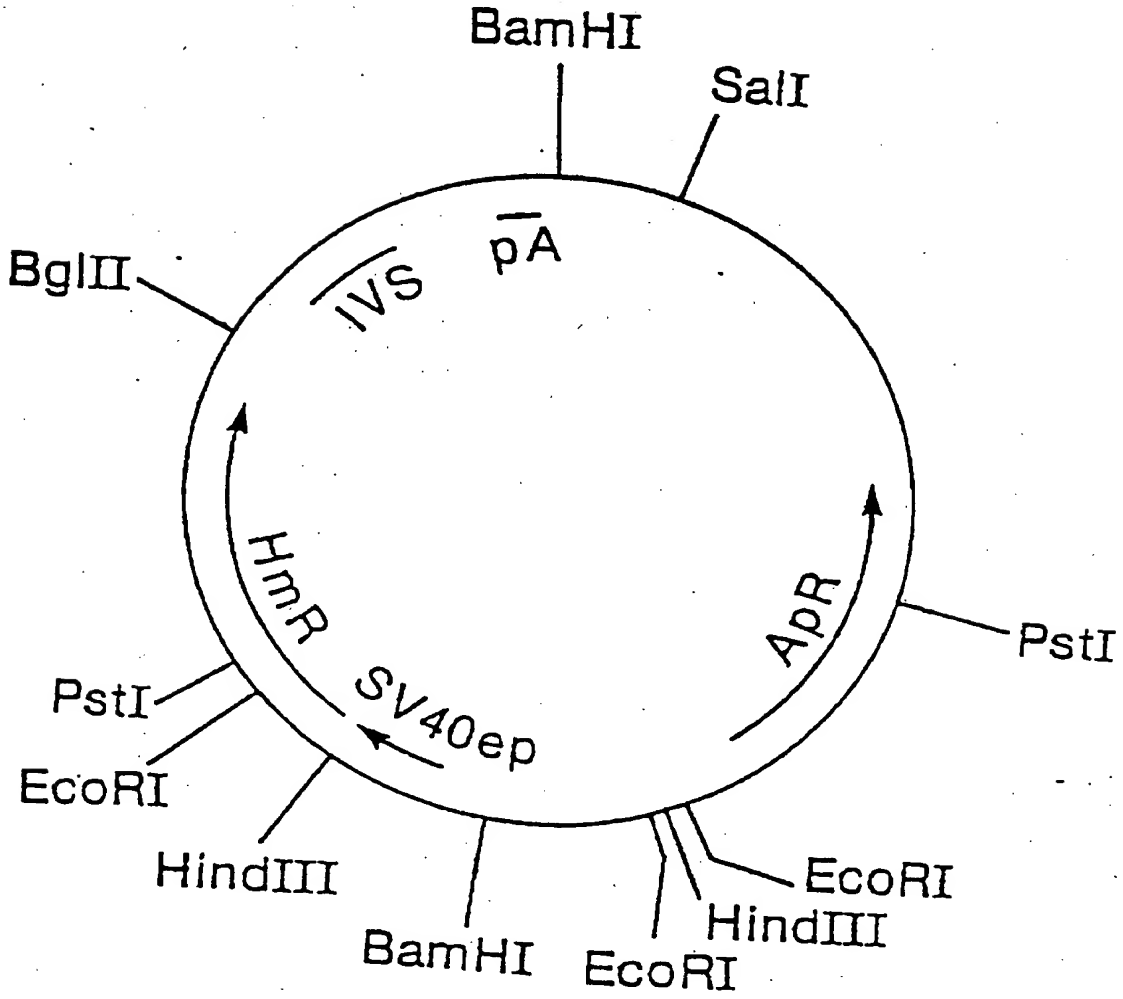

BamHI
SalI
pA
IVS
BglII
HmR
SV40ep
ApR
PstI
PstI
EcoRI
HindIII
BamHI
EcoRI
HindIII
EcoRI

Figure 13

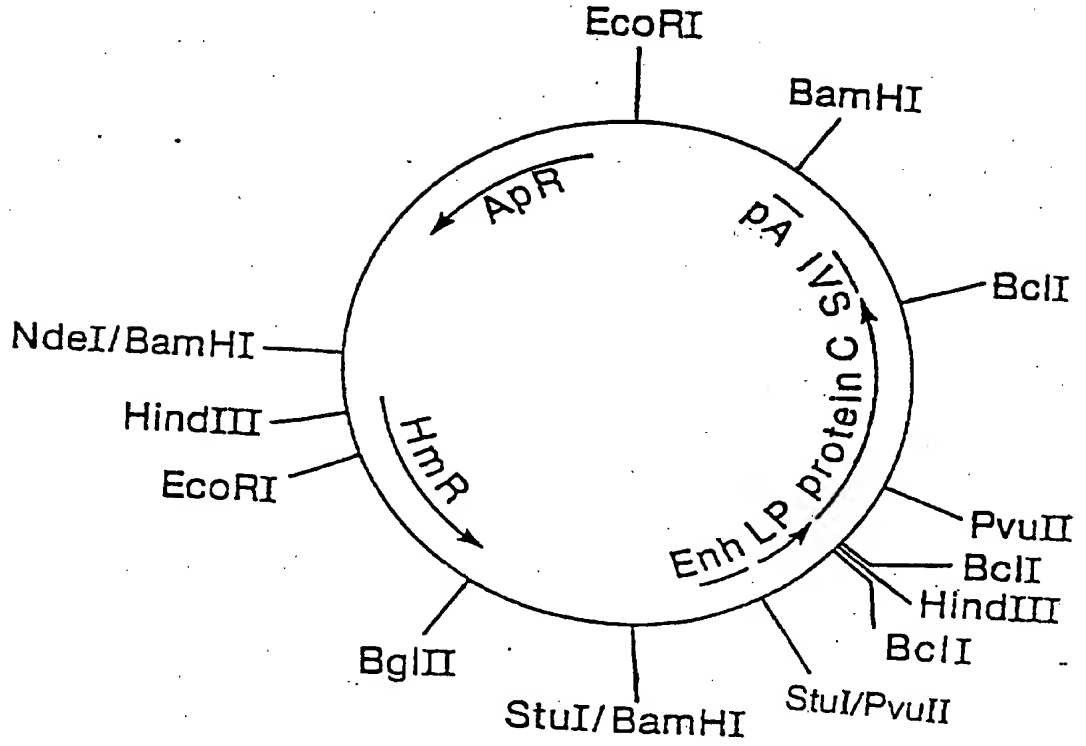
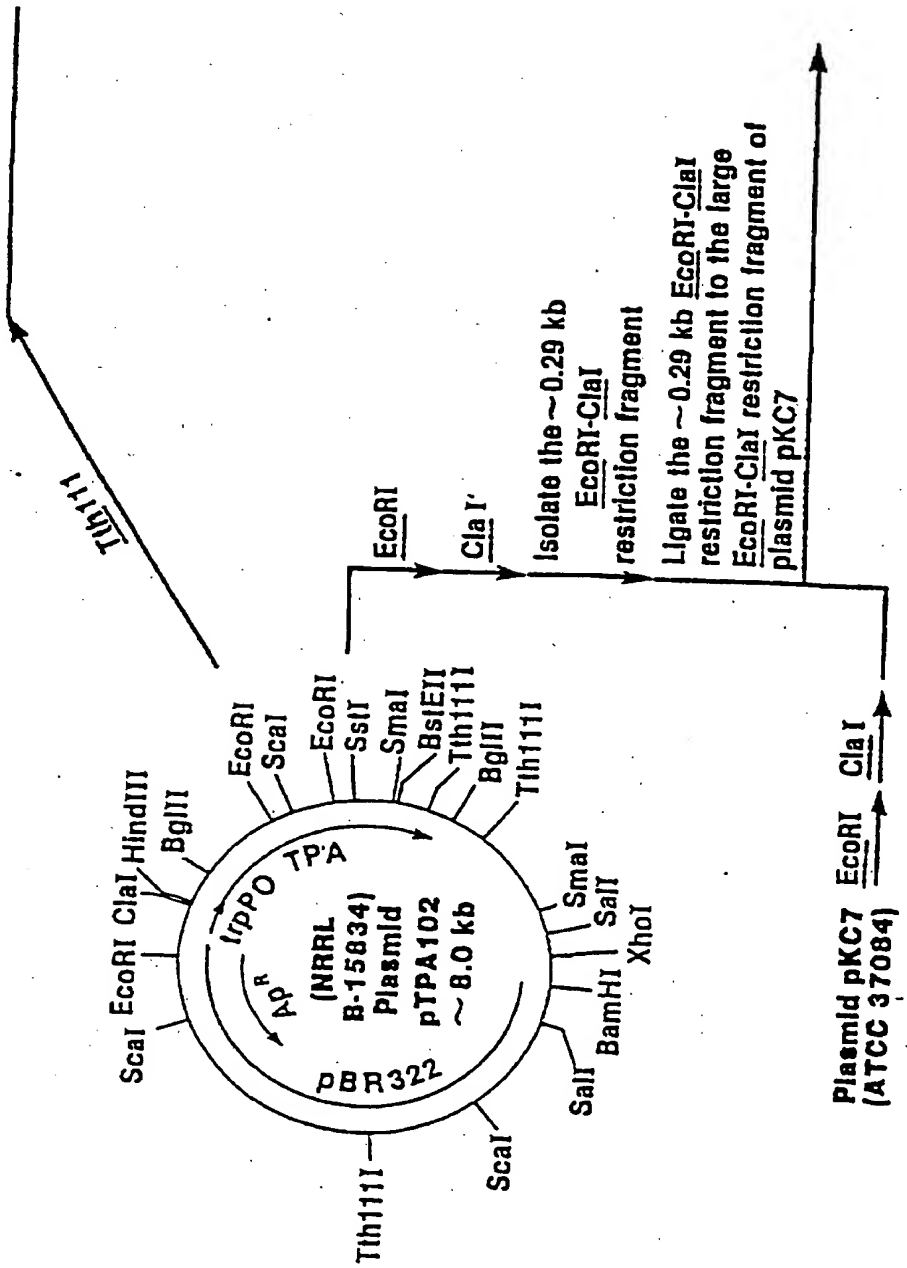

EcoRI
BamHI
BclI
PvuII
BclI
HindIII
BclI
StuI/PvuII
StuI/BamHI
BglII
EcoRI
HindIII
NdeI/BamHI
ApR
pA IVS
protein C LP
Enh
HmR

Figure 14 A


Tth111I
EcoRI
Cla I
Isolate the ~0.29 kb EcoRI-ClaI restriction fragment
Ligate the ~0.29 kb EcoRI-ClaI restriction fragment to the large EcoRI-ClaI restriction fragment of plasmid pKC7
Plasmid pKC7 (ATCC 37084)
EcoRI
Cla I
TPA
IrpO
Ap^R
(NRRL B-15834) Plasmid pTPA102 ~8.0 kb
pBR322
EcoRI
ScaI
EcoRI
SstI
SmaI
BstEII
Tth111I
BglII
Tth111I
SmaI
SalI
XhoI
BamHI
SalI
ScaI
Tth111I
ScaI
EcoRI
ClaI
HindIII
BglII

5,681,932

Figure 14 B

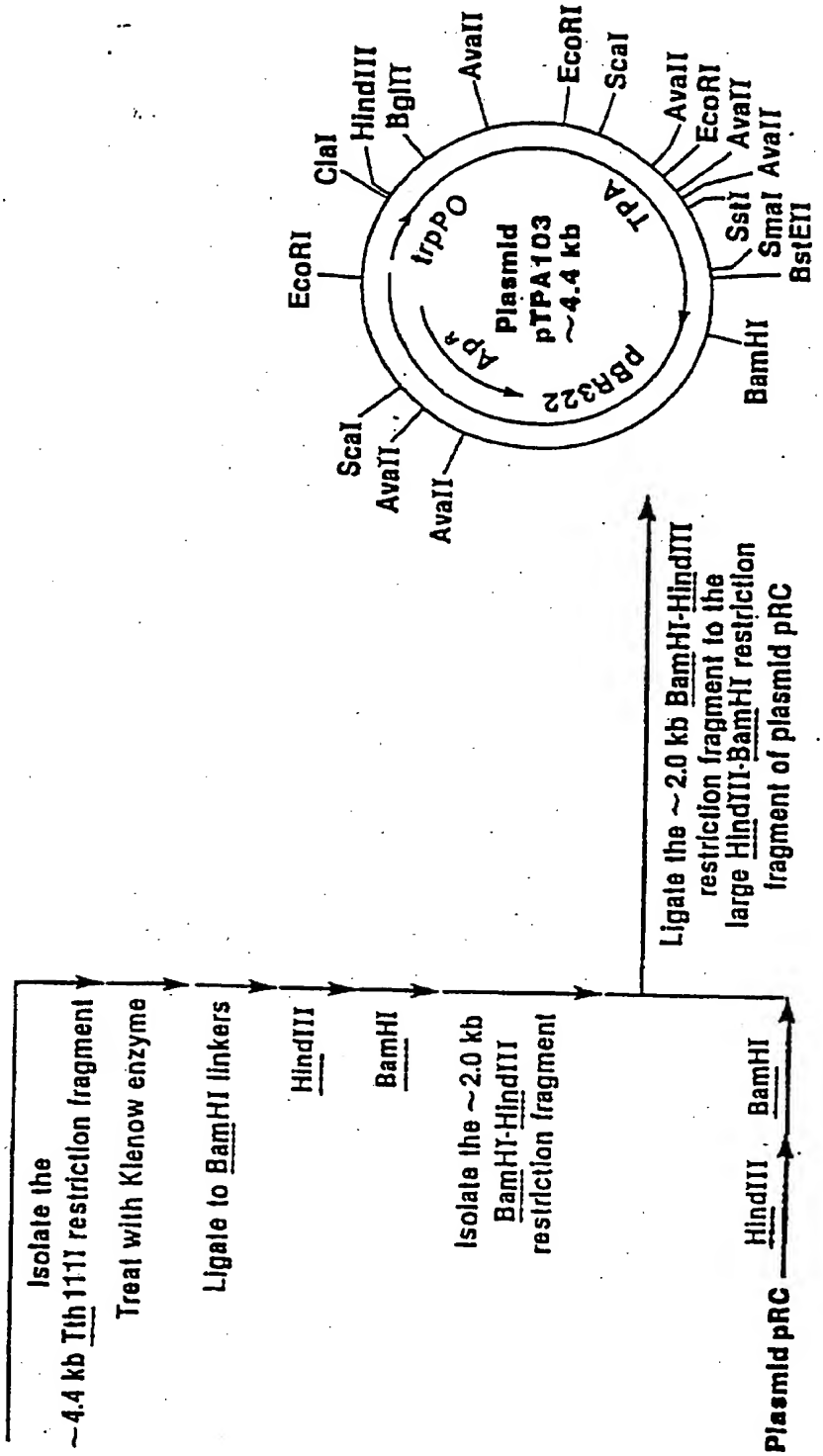


Figure 14 C

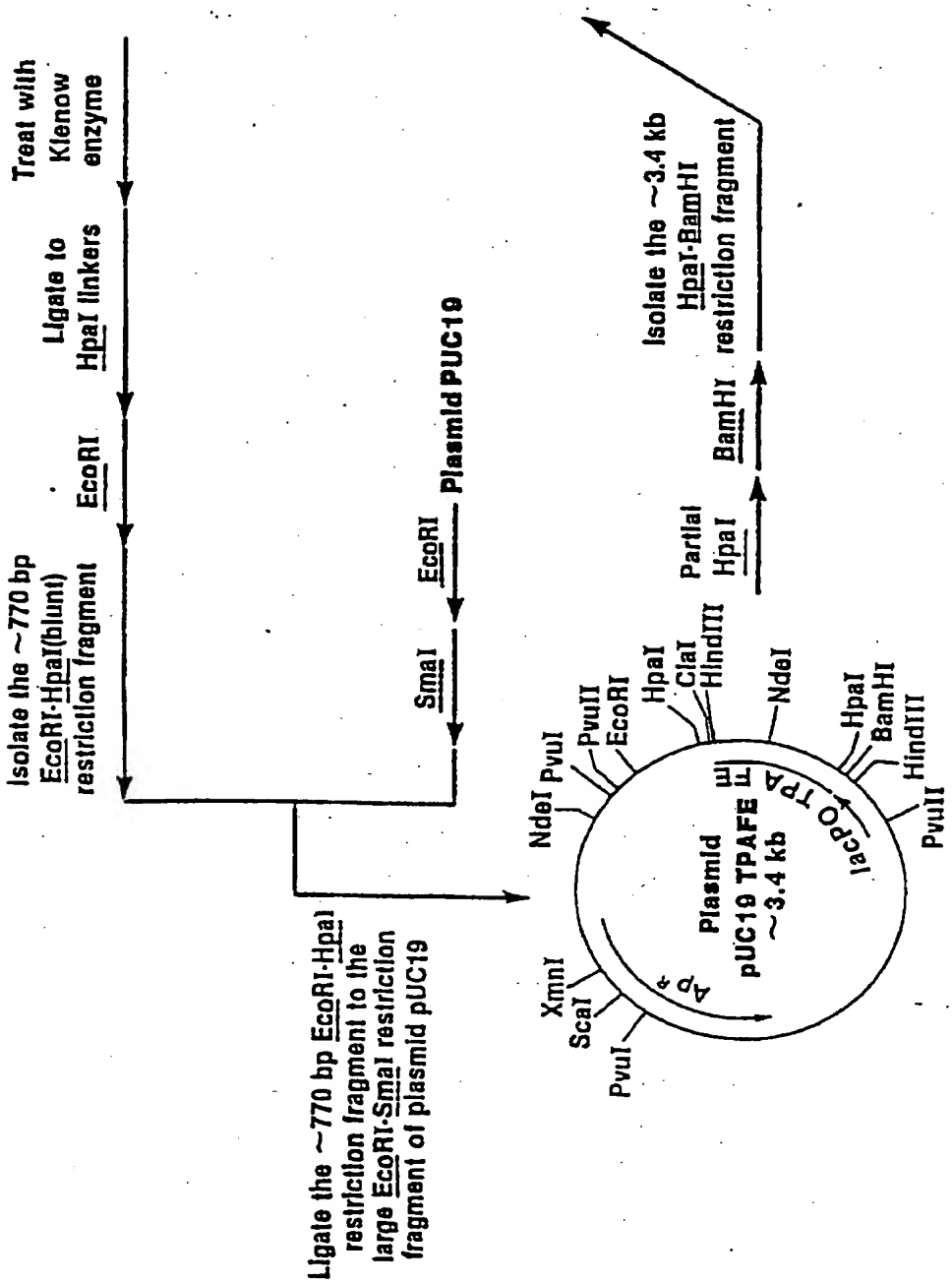

Isolate the ~770 bp EcoRI-HpaI(blunt) restriction fragment
EcoRI
Ligate to HpaI linkers
Treat with Klenow enzyme
SmaI
EcoRI
Plasmid PUC19
Ligate the ~770 bp EcoRI-HpaI restriction fragment to the large EcoRI-SmaI restriction fragment of plasmid pUC19
Plasmid pUC19 TPAFE ~3.4 kb
Ap^R
lacP/O
TPA
FE
NdeI
PvuI
PvuII
EcoRI
HpaI
ClaI
HindIII
NdeI
HpaI
BamHI
HindIII
PvuII
PvuI
ScaI
XmnI
PartIal HpaI
BamHI
Isolate the ~3.4 kb HpaI-BamHI restriction fragment

Figure 14 D

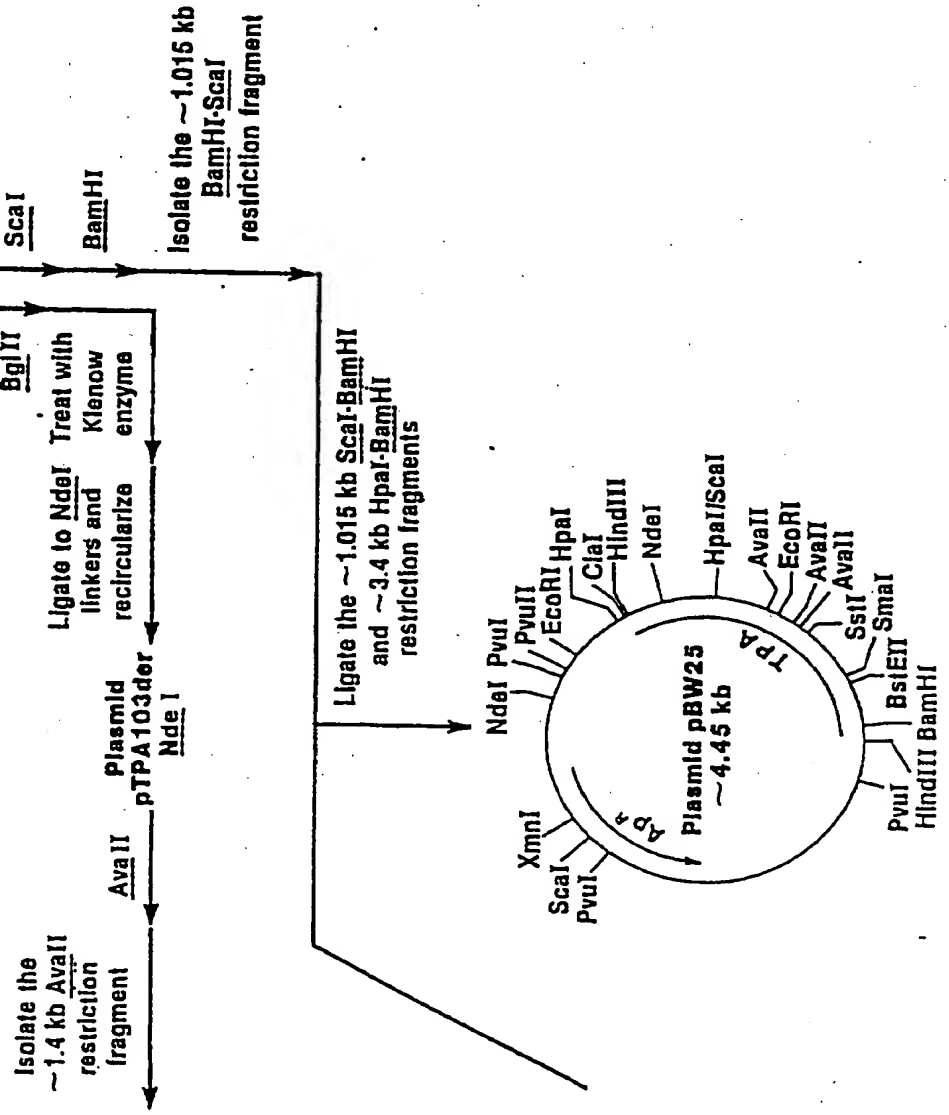

ScaI
BamHI
Isolate the ~1.015 kb BamHI-ScaI restriction fragment
BglII
Treat with Klenow enzyme
Ligate to NdeI linkers and recircularize
Plasmid pTPA103der NdeI
AvaII
Isolate the ~1.4 kb AvaII restriction fragment
Ligate the ~1.015 kb ScaI-BamHI and ~3.4 kb HpaI-BamHI restriction fragments
NdeI PvuI PvuII EcoRI HpaI ClaI HindIII NdeI HpaI/ScaI AvaII EcoRI AvaII AvaII SstI SmaI BstEII BamHI HindIII PvuI XmnI ScaI PvuI
Plasmid pBW25 ~4.45 kb
TPA
Ap

Figure 14 E

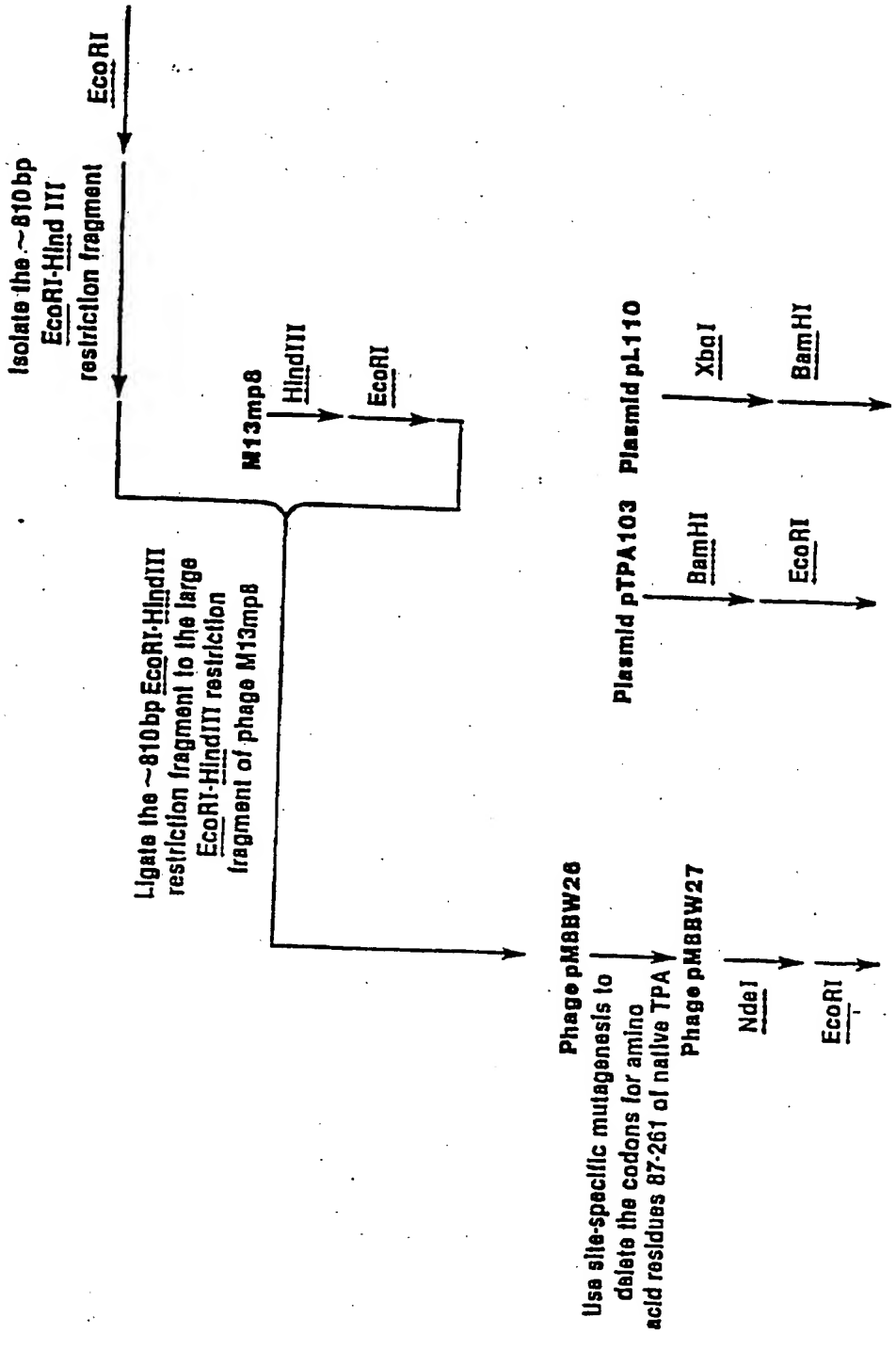

EcoRI
Isolate the ~810 bp EcoRI-Hind III restriction fragment
M13mp8
HindIII
EcoRI
Ligate the ~810 bp EcoRI-HindIII restriction fragment to the large EcoRI-HindIII restriction fragment of phage M13mp8
Plasmid pL110
XbaI
BamHI
Plasmid pTPA103
BamHI
EcoRI
Phage pM8BW26
Use site-specific mutagenesis to delete the codons for amino acid residues 87-261 of native TPA
Phage pM8BW27
NdeI
EcoRI

Figure 14 F

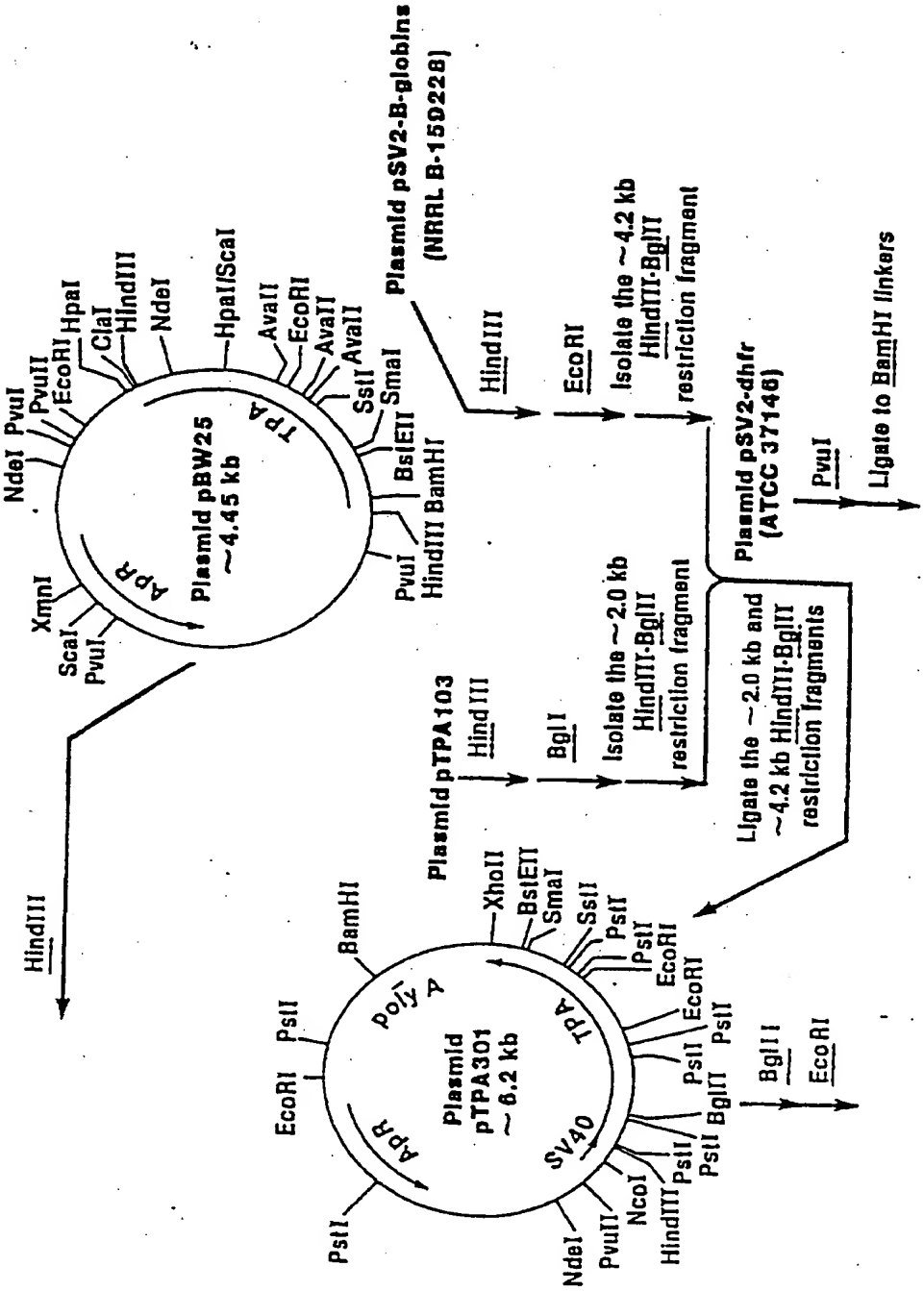

Plasmid pBW25
~4.45 kb
TPA
APR
NdeI PvuI PvuII EcoRI HpaI ClaI HindIII NdeI HpaI/ScaI AvaII EcoRI AvaII AvaII SstII SmaI BstEII BamHI HindIII PvuI XmnI ScaI PvuI
HindIII
Plasmid pSV2-B-globins
(NRRL B-15928)
HindIII
EcoRI
Isolate the ~4.2 kb
HindIII-BglII
restriction fragment
Plasmid pSV2-dhfr
(ATCC 37146)
PvuI
Ligate to BamHI linkers
Plasmid pTPA103
HindIII
BglI
Isolate the ~2.0 kb
HindIII-BglII
restriction fragment
Ligate the ~2.0 kb and
~4.2 kb HindIII-BglII
restriction fragments
Plasmid
pTPA301
~6.2 kb
APR
Poly A
TPA
SV40
BamHI XhoII BstEII SmaI SstII PstI PstI EcoRI EcoRI PstI PstI BglII EcoRI PstI PstI BglII PstI NcoI HindIII PvuII NdeI PstI EcoRI
BglII
EcoRI

Figure 14 G

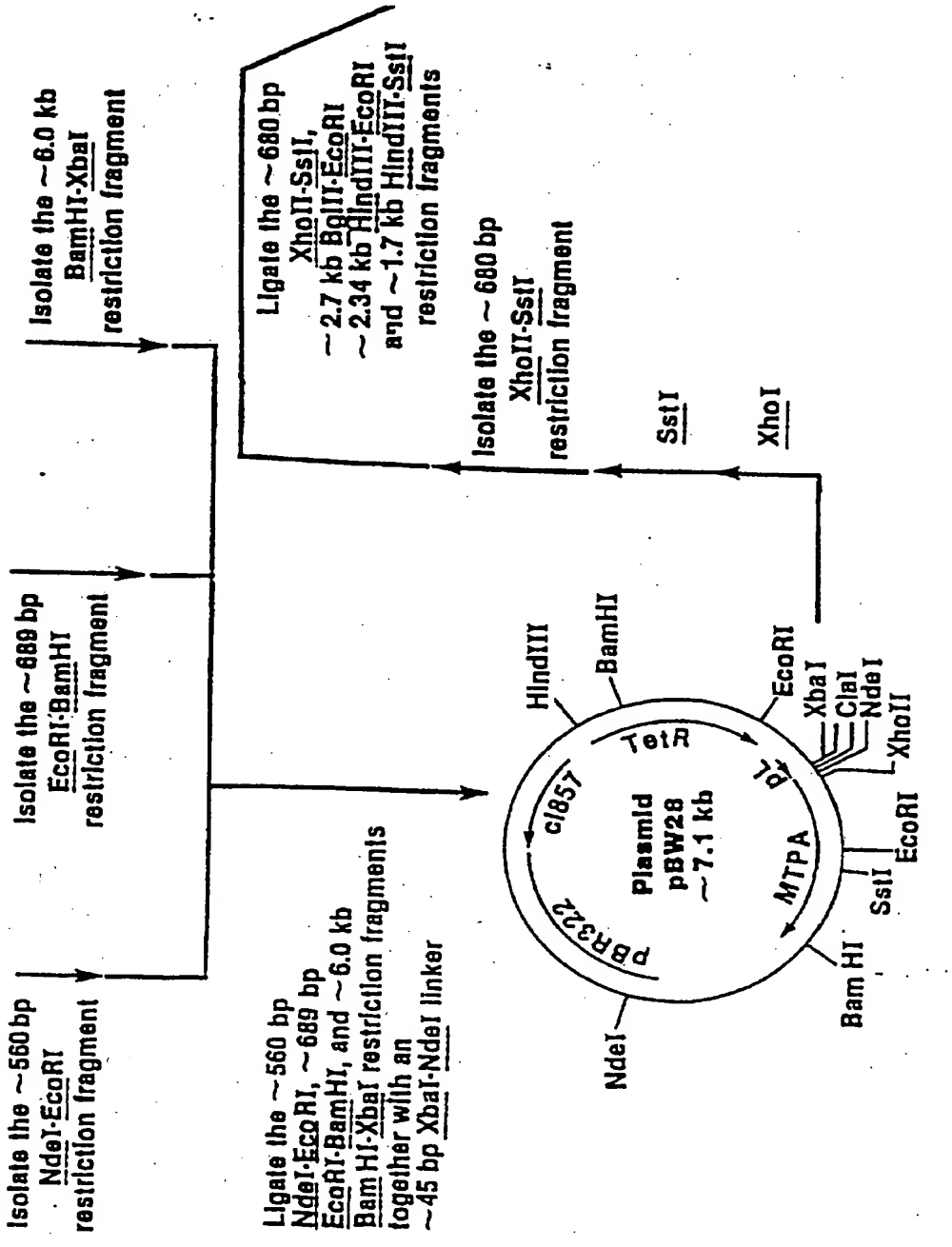

Isolate the ~6.0 kb BamHI-XbaI restriction fragment
Ligate the ~680 bp XhoII-SstI, ~2.7 kb BglII-EcoRI ~2.34 kb HindIII-EcoRI and ~1.7 kb HindIII-SstI restriction fragments
Isolate the ~680 bp XhoII-SstI restriction fragment
SstI
XhoI
Isolate the ~689 bp EcoRI-BamHI restriction fragment
Isolate the ~560 bp NdeI-EcoRI restriction fragment
Ligate the ~560 bp NdeI-EcoRI, ~689 bp EcoRI-BamHI, and ~6.0 kb BamHI-XbaI restriction fragments together with an ~45 bp XbaI-NdeI linker
HindIII
BamHI
EcoRI
XbaI
ClaI
NdeI
XhoII
TetR
cI857
Plasmid pBW28 ~7.1 kb
PL
MTPA
pBR322
SstI
EcoRI
BamHI
NdeI

Figure 14 H

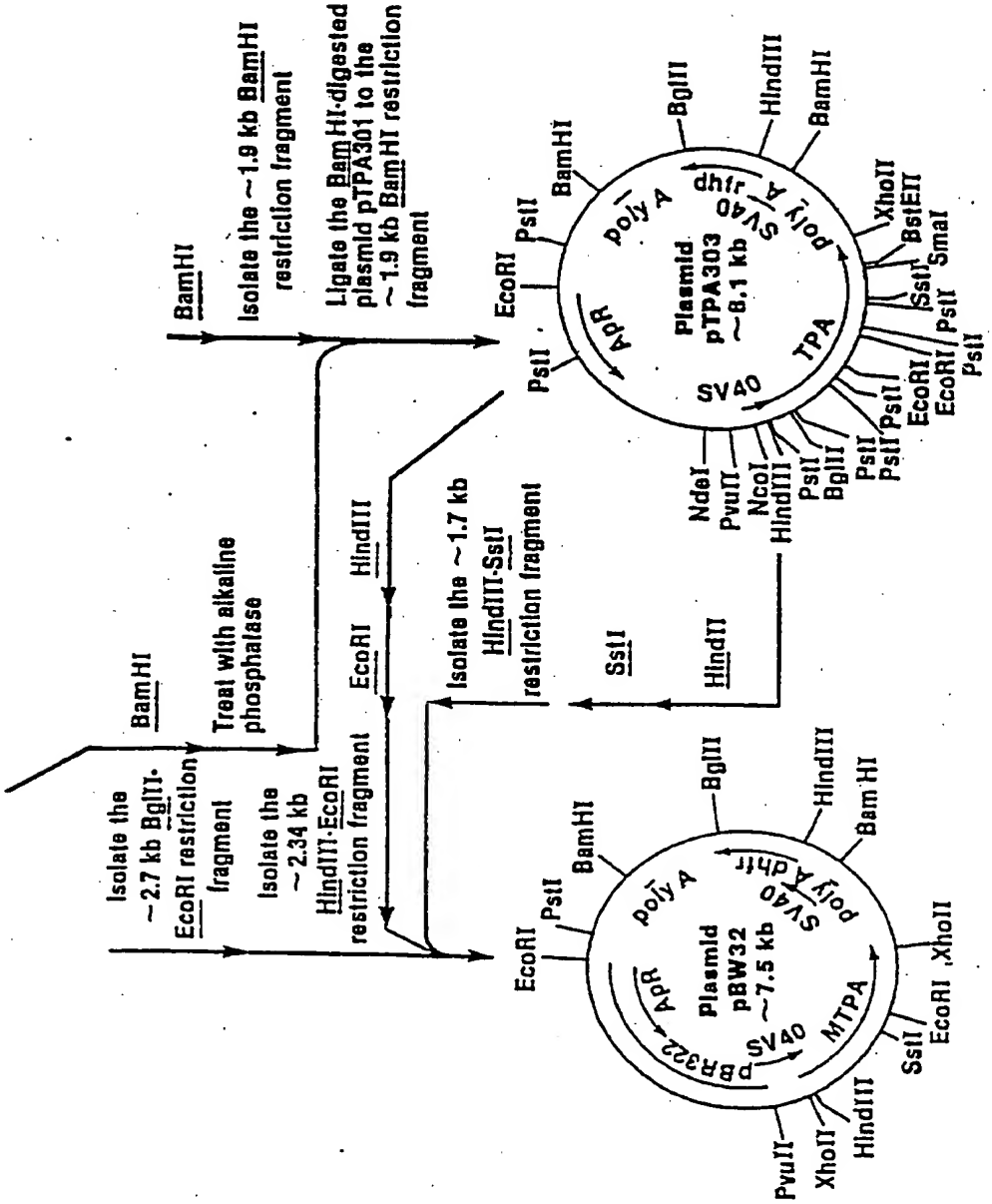

BamHI
Isolate the ~1.9 kb BamHI restriction fragment
Ligate the BamHI-digested plasmid pTPA301 to the ~1.9 kb BamHI restriction fragment
Plasmid pTPA303 ~8.1 kb
Treat with alkaline phosphatase
Isolate the ~2.7 kb BglII-EcoRI restriction fragment
Isolate the ~2.34 kb HindIII-EcoRI restriction fragment
EcoRI
HindIII
Isolate the ~1.7 kb HindIII-SstI restriction fragment
SstI
HindII
Plasmid pBW32 ~7.5 kb

Figure 14 I

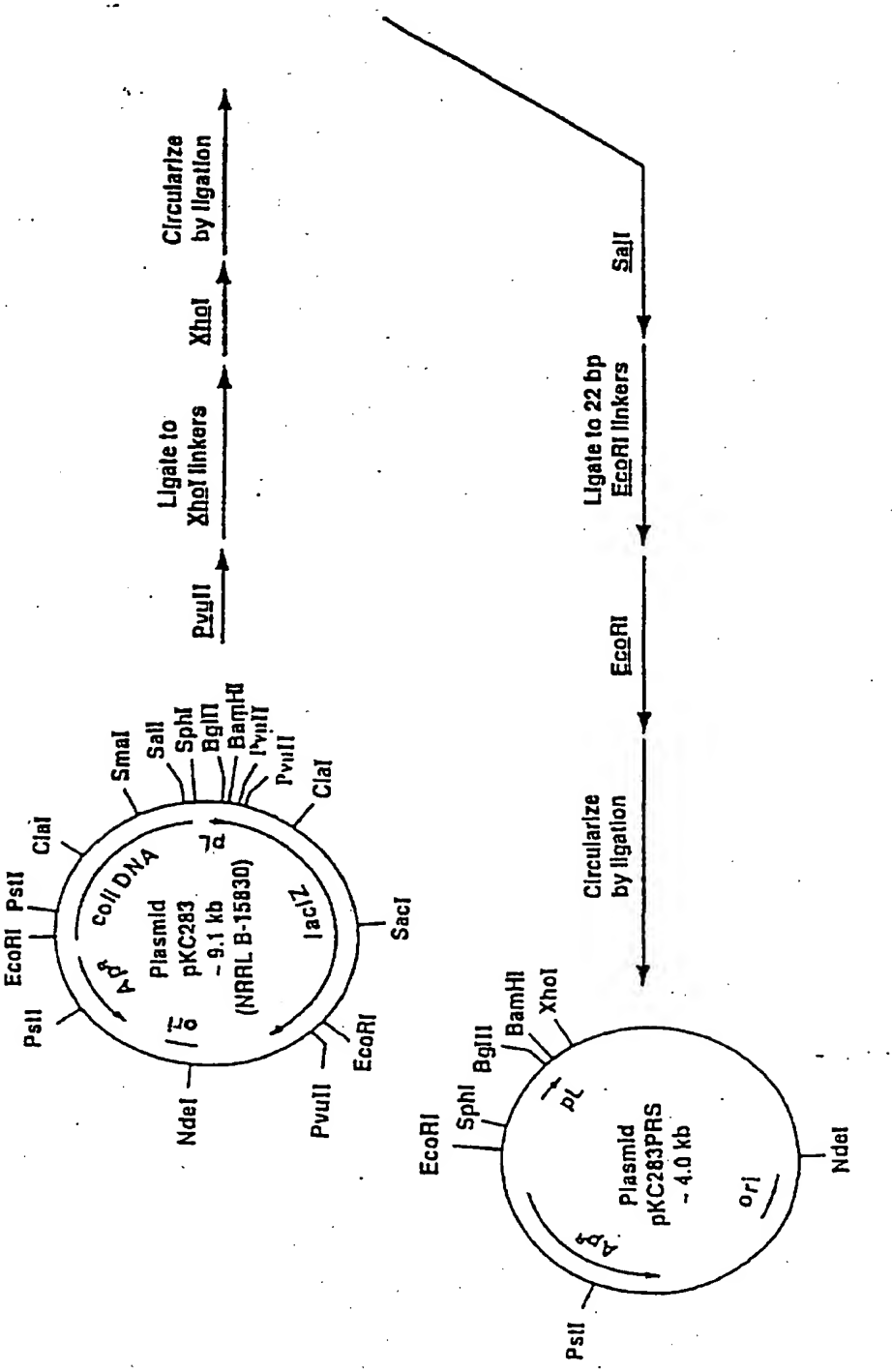

Circularize by ligation
Xhol
Ligate to Xhol linkers
PvuII
Circularize by ligation
EcoRI
Ligate to 22 bp EcoRI linkers
SalI
Plasmid pKC283 - 9.1 kb (NRRL B-15830)
Plasmid pKC283PRS - 4.0 kb
EcoRI
PstI
ClaI
SmaI
SalI
SphI
BglII
BamHI
PvuII
ClaI
SacI
EcoRI
PvuII
NdeI
PstI
EcoRI
SphI
BglII
BamHI
XhoI
NdeI
PstI

Figure 14 J

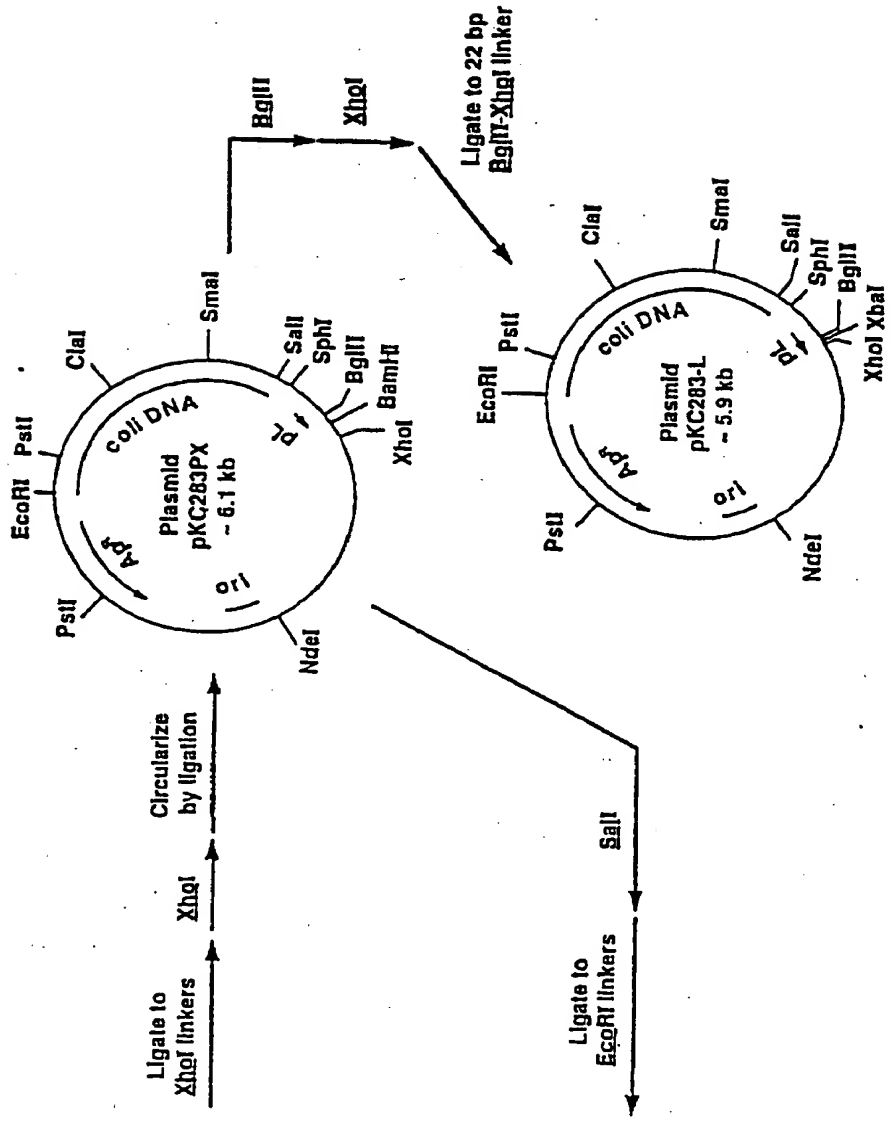

BglII
XhoI
Ligate to 22 bp BglII-XhoI linker
ClaI
SmaI
SalI
SphI
BglII
XhoI XbaI
PstI
EcoRI
coli DNA
Plasmid pKC283-L ~ 5.9 kb
ori
NdeI
ClaI
SmaI
SalI
SphI
BglII
BamHI
XhoI
EcoRI
PstI
coli DNA
Plasmid pKC283PX ~ 6.1 kb
ori
NdeI
Circularize by ligation
XhoI
Ligate to XhoI linkers
SalI
Ligate to EcoRI linkers

Figure 14 K

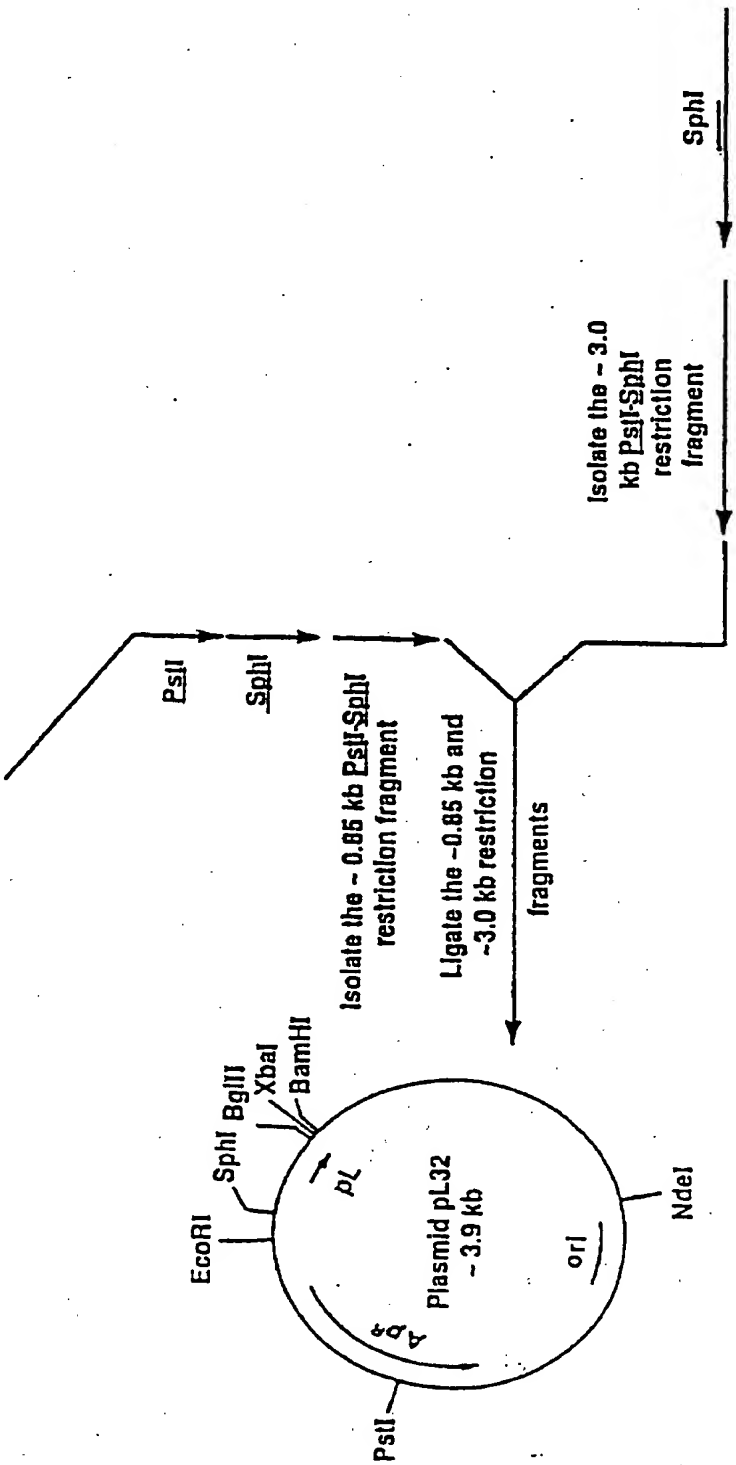

SphI
Isolate the ~ 3.0 kb PstI-SphI restriction fragment
PstI
SphI
Isolate the ~ 0.85 kb PstI-SphI restriction fragment
Ligate the ~0.85 kb and ~3.0 kb restriction fragments
BamHI
XbaI
BglII
SphI
EcoRI
pL
Plasmid pL32 ~ 3.9 kb
ori
NdeI
Ap
PstI

Figure 14 L

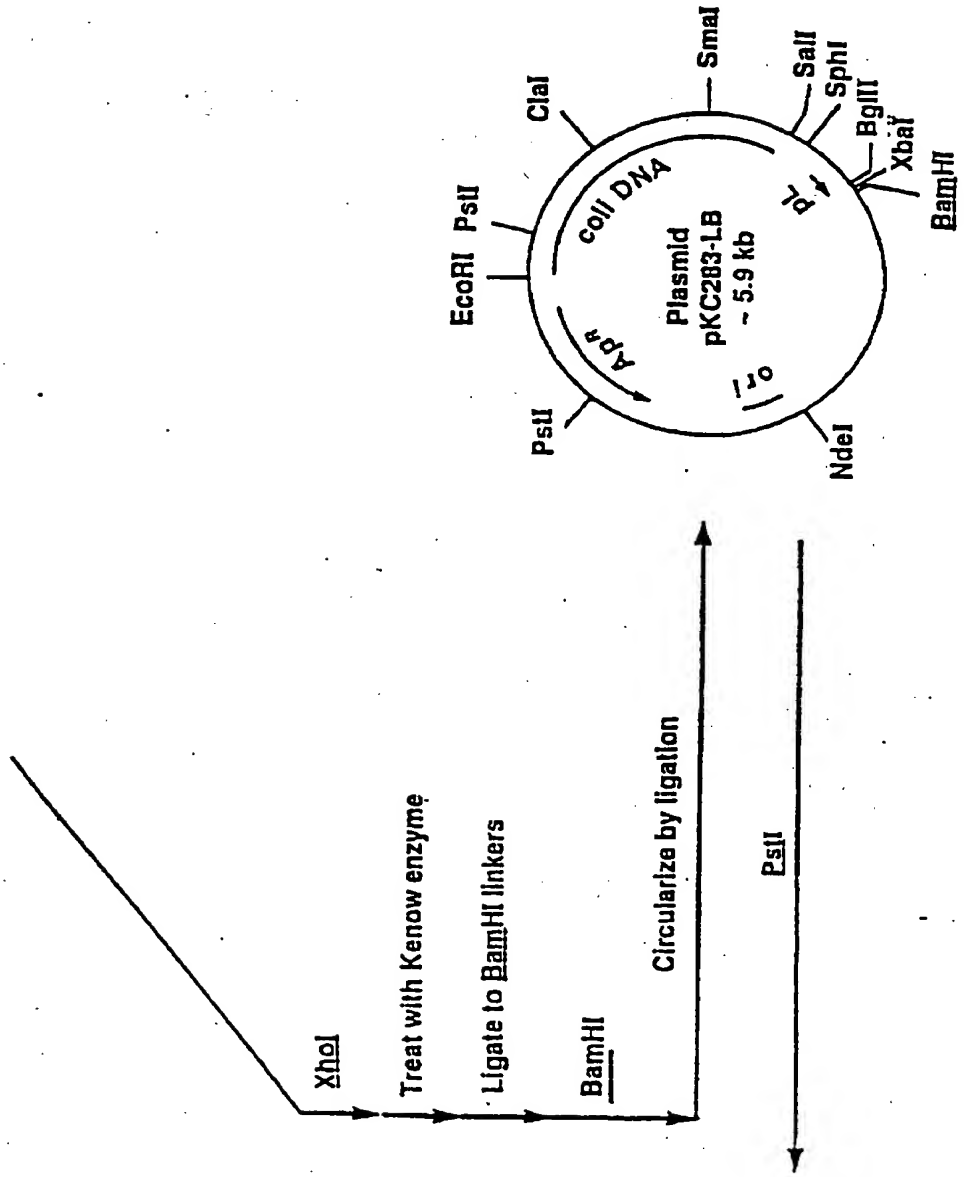

XhoI
Treat with Kenow enzyme
Ligate to BamHI linkers
BamHI
Circularize by ligation
PstI
Plasmid
pKC283-LB
~ 5.9 kb
coll DNA
ori
NdeI
PstI
EcoRI
ClaI
SmaI
SalI
SphI
BglII
XbaI
BamHI

Figure 14 M

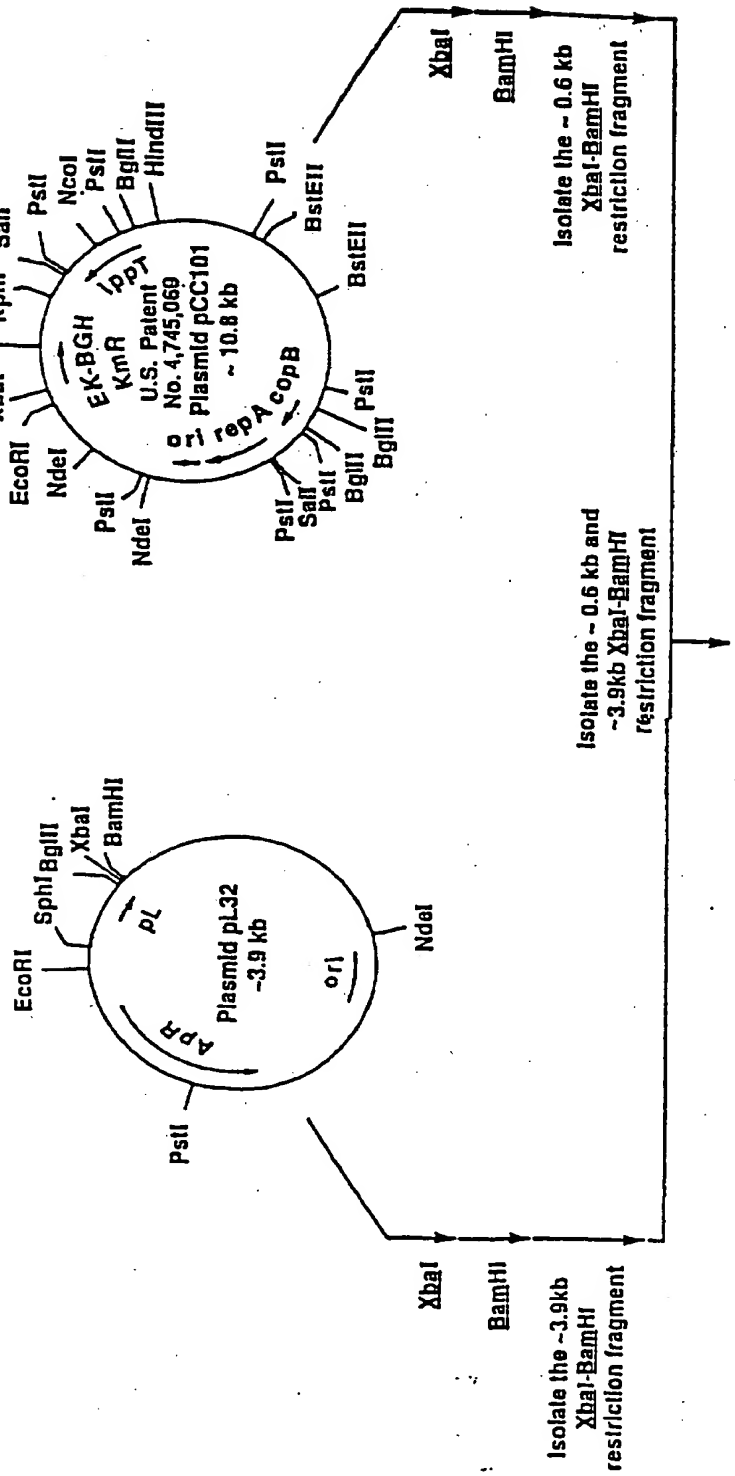

Plasmid pC101
~10.8 kb
U.S. Patent
No. 4,745,069
EK-BGH
KmR
lpdT
repA
copB
ori
PstI
BstEII
BglII
SalI
NdeI
EcoRI
XbaI
BamHI
KpnI
SalI
NcoI
HindIII
Plasmid pL32
~3.9 kb
pL
pR
APR
ori
SphI
Isolate the ~ 0.6 kb
XbaI-BamHI
restriction fragment
Isolate the ~ 0.6 kb and
~3.9kb XbaI-BamHI
restriction fragment
Isolate the ~3.9kb
XbaI-BamHI
restriction fragment

Figure 14 N

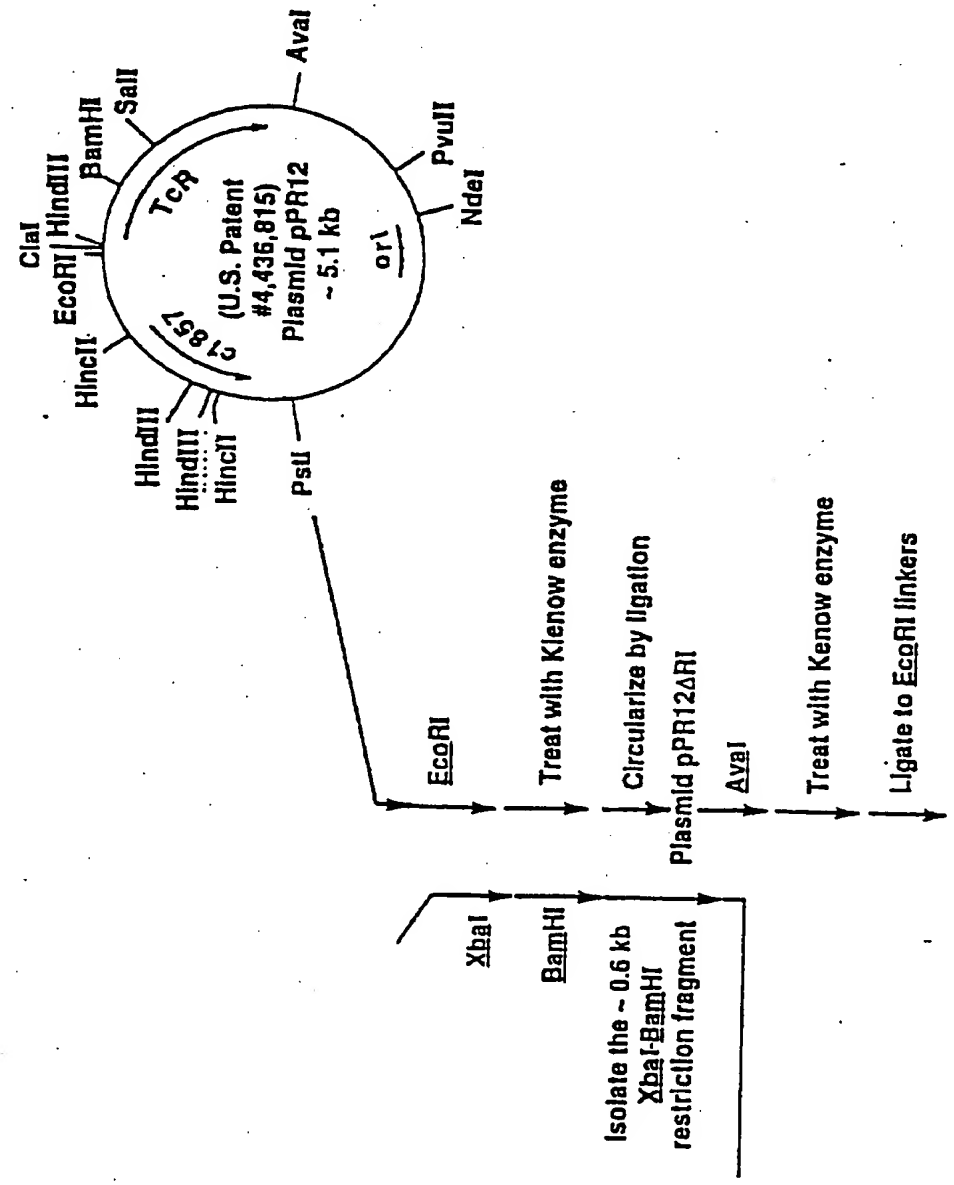

AvaI
PvuII
NdeI
ori
(U.S. Patent #4,436,815) Plasmid pPR12 ~ 5.1 kb
Tcr
c1857
SalI
BamHI
HindIII
ClaI
EcoRI
HincII
HindIII
HindIII
HincII
PstI
EcoRI
Treat with Klenow enzyme
Circularize by ligation
Plasmid pPR12ΔRI
AvaI
Treat with Kenow enzyme
Ligate to EcoRI linkers
XbaI
BamHI
Isolate the ~ 0.6 kb XbaI-BamHI restriction fragment

Figure 14 O

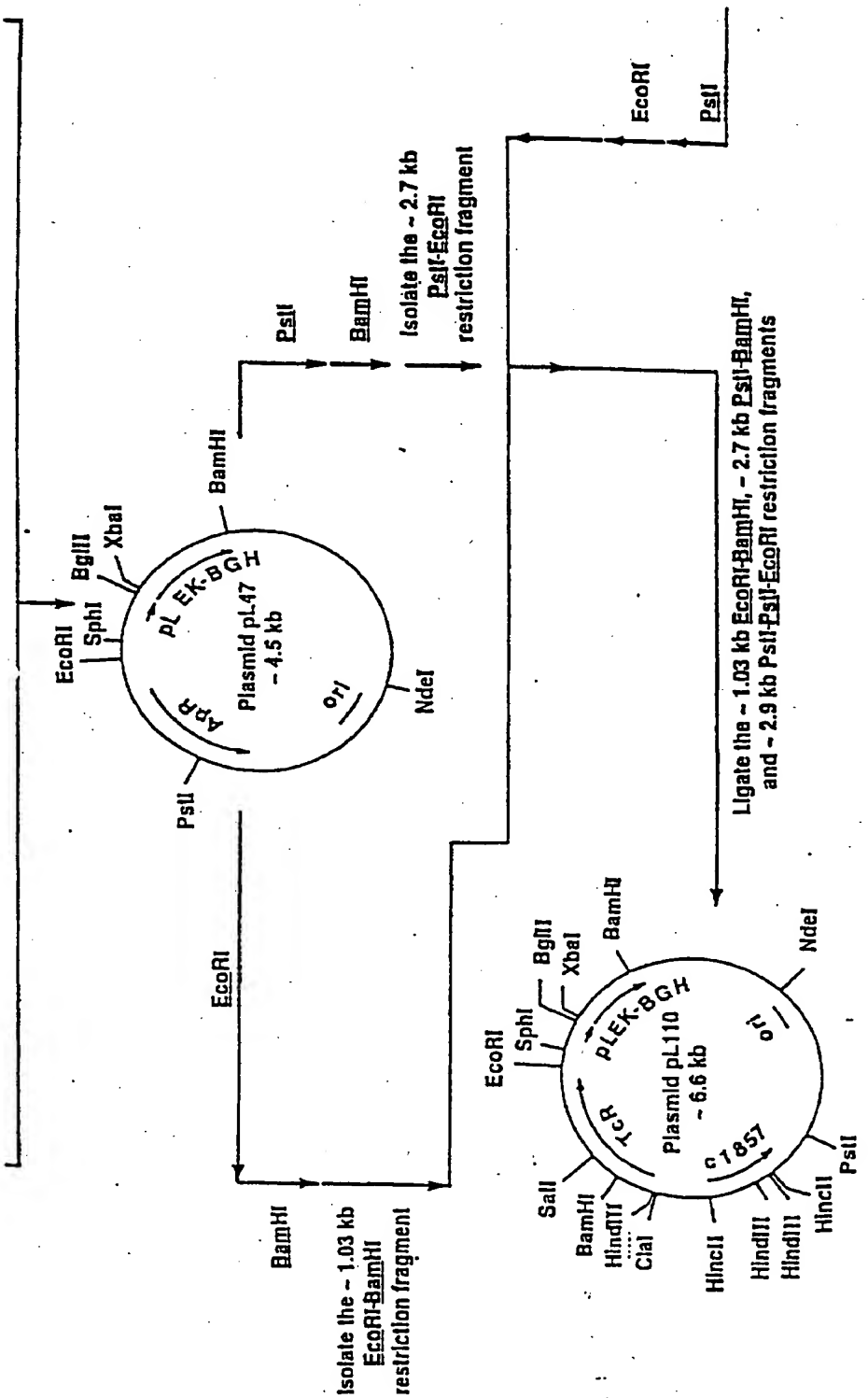

EcoRI
PstI
Isolate the ~ 2.7 kb PstI-EcoRI restriction fragment
BamHI
PstI
BamHI
BglII
XbaI
SphI
EcoRI
pL EK-BGH
Plasmid pL47 ~ 4.5 kb
ApR
ori
NdeI
PstI
EcoRI
BamHI
Isolate the ~ 1.03 kb EcoRI-BamHI restriction fragment
Ligate the ~ 1.03 kb EcoRI-BamHI, ~ 2.7 kb PstI-BamHI, and ~ 2.9 kb PstI-PstI-EcoRI restriction fragments
BamHI
BglII
XbaI
SphI
EcoRI
pLEK-BGH
Plasmid pL110 ~ 6.6 kb
TcR
cI857
ori
NdeI
SalI
BamHI
HindIII
ClaI
HincII
HindIII
HindIII
HincII
PstI

Figure 14 P

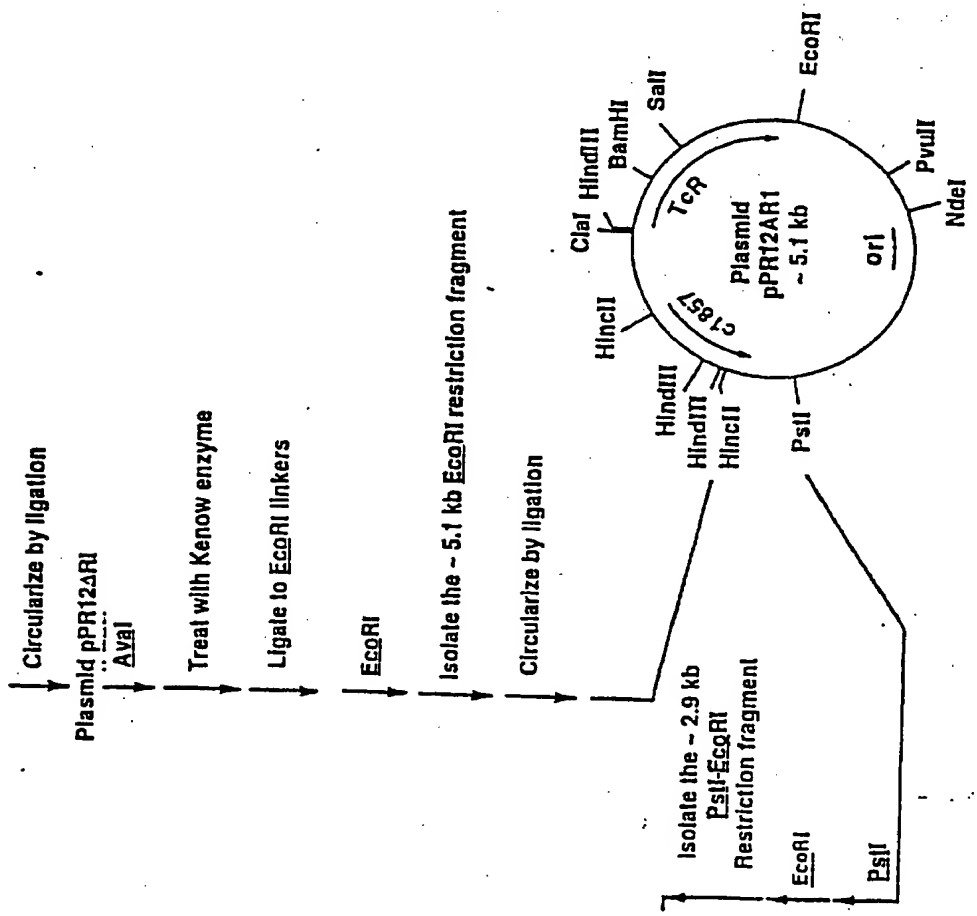

Circularize by ligation
Plasmid pPR12ΔRI
AvaI
Treat with Klenow enzyme
Ligate to EcoRI linkers
EcoRI
Isolate the ~ 5.1 kb EcoRI restriction fragment
Circularize by ligation
Isolate the ~ 2.9 kb PstI-EcoRI Restriction fragment
EcoRI
PstI
Plasmid pPR12AR1 ~ 5.1 kb
TcR
c1857
ori
EcoRI
SalI
BamHI
HindIII
ClaI
HincII
HindIII
HindIII
HincII
PstI
PvuII
NdeI

Figure 15

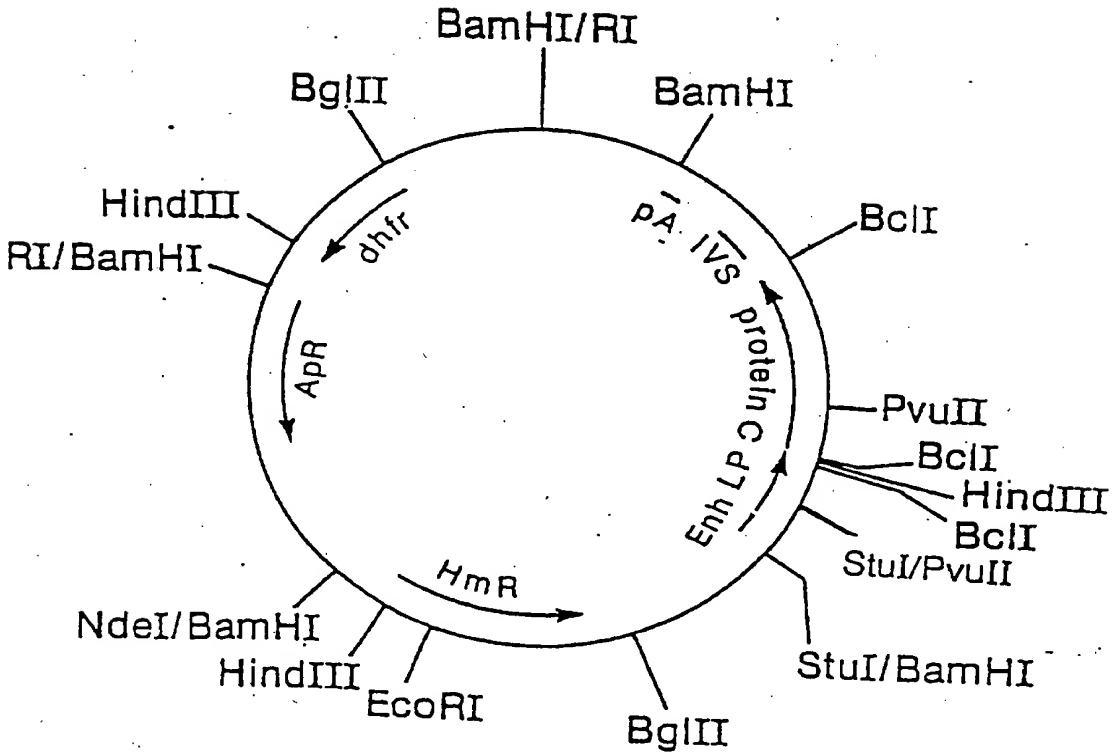

BamHI/RI
BglII
BamHI
HindIII
RI/BamHI
dhfr
pA
IVS
BclI
ApR
protein C
PvuII
BclI
HindIII
BclI
Enh LP
StuI/PvuII
HmR
NdeI/BamHI
HindIII
EcoRI
BglII
StuI/BamHI

Figure 16

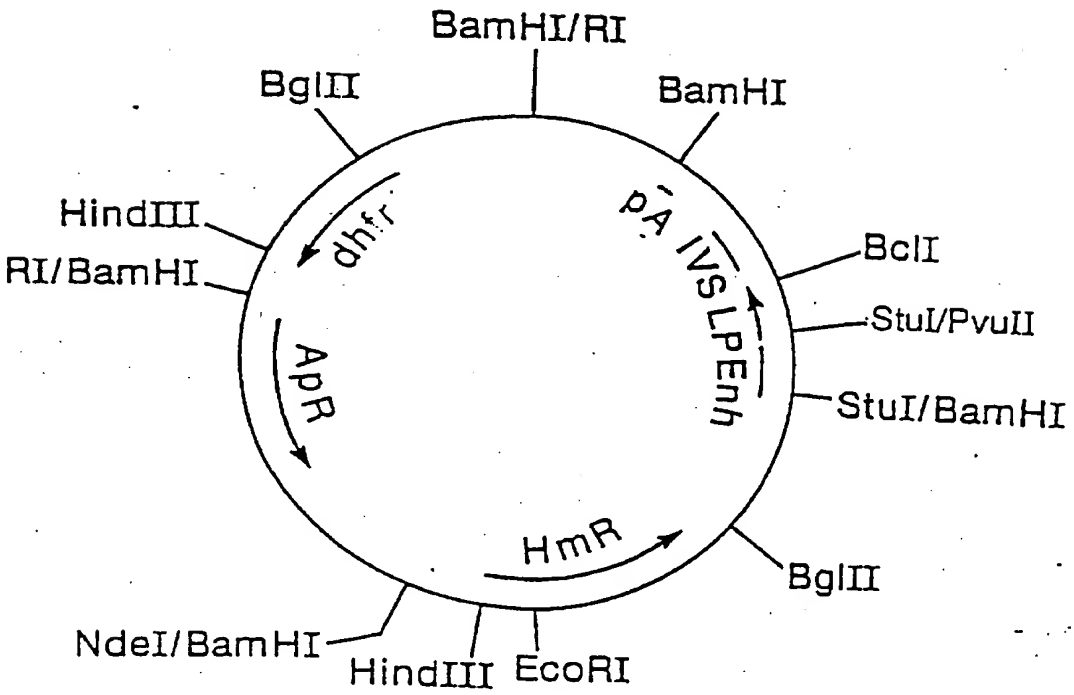

BamHI/RI
BglII
BamHI
HindIII
RI/BamHI
dhfr
pA
IVS
LPEnh
BclI
StuI/PvuII
StuI/BamHI
ApR
HmR
BglII
NdeI/BamHI
HindIII
EcoRI

Figure 17

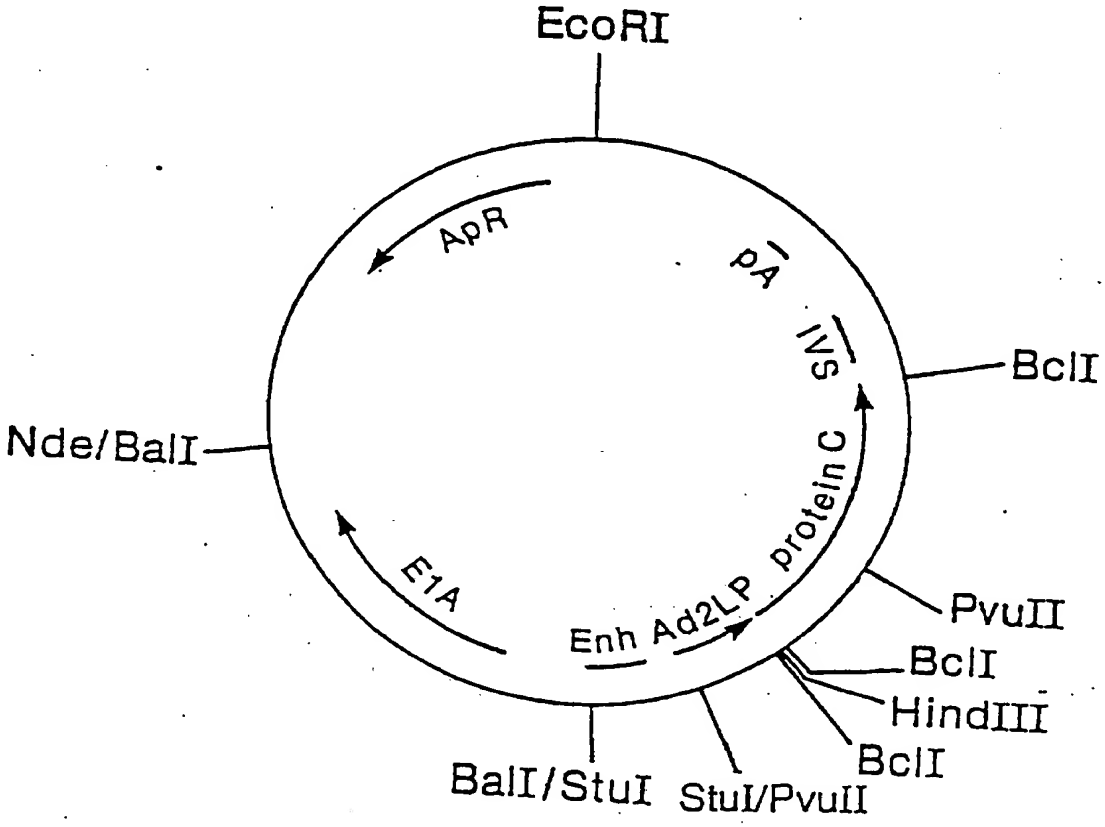

EcoRI
ApR
pA
IVS
BclI
Nde/BalI
protein C
E1A
Enh Ad2LP
PvuII
BclI
HindIII
BclI
BalI/StuI
StuI/PvuII

Figure 18

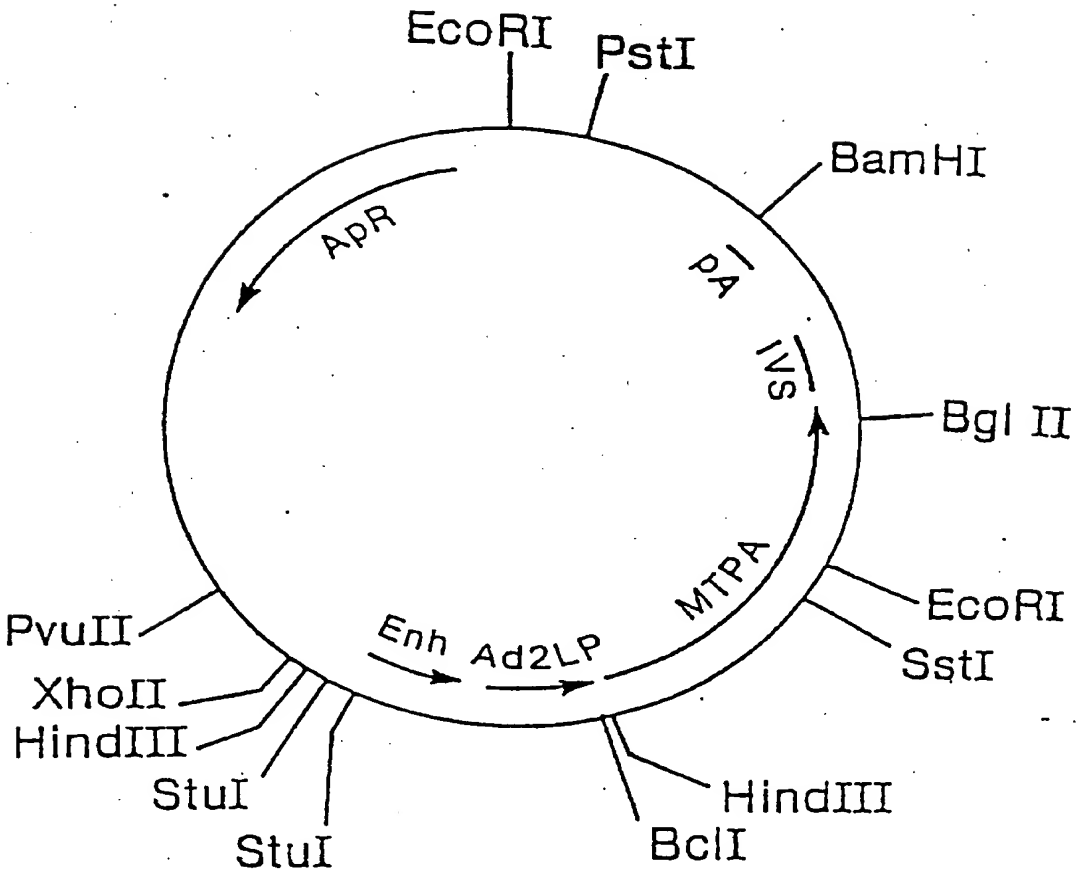

EcoRI
PstI
BamHI
ApR
pA
IVS
Bgl II
EcoRI
SstI
MTPA
Enh
Ad2LP
PvuII
XhoII
HindIII
StuI
StuI
HindIII
BclI

Figure 19

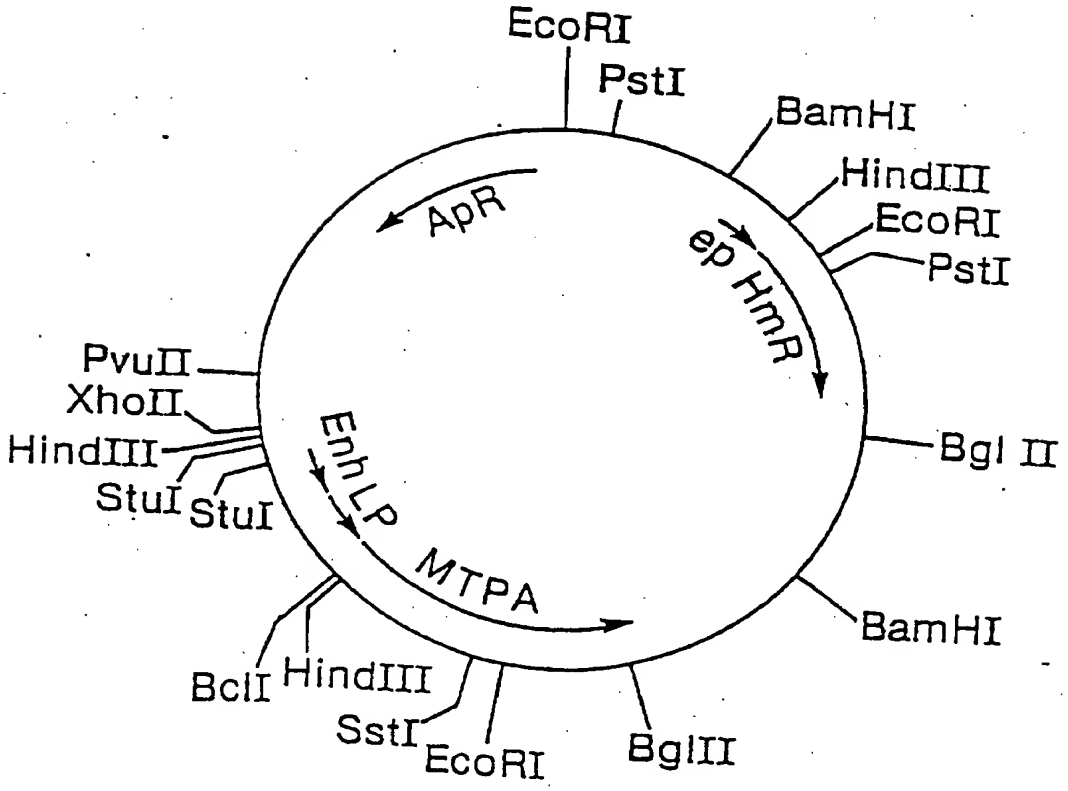

EcoRI
PstI
BamHI
HindIII
EcoRI
PstI
ApR
eP HmR
PvuII
XhoII
HindIII
StuI
StuI
EnhLP
MTPA
BglII
BamHI
BclI
HindIII
SstI
EcoRI
BglII

Figure 20

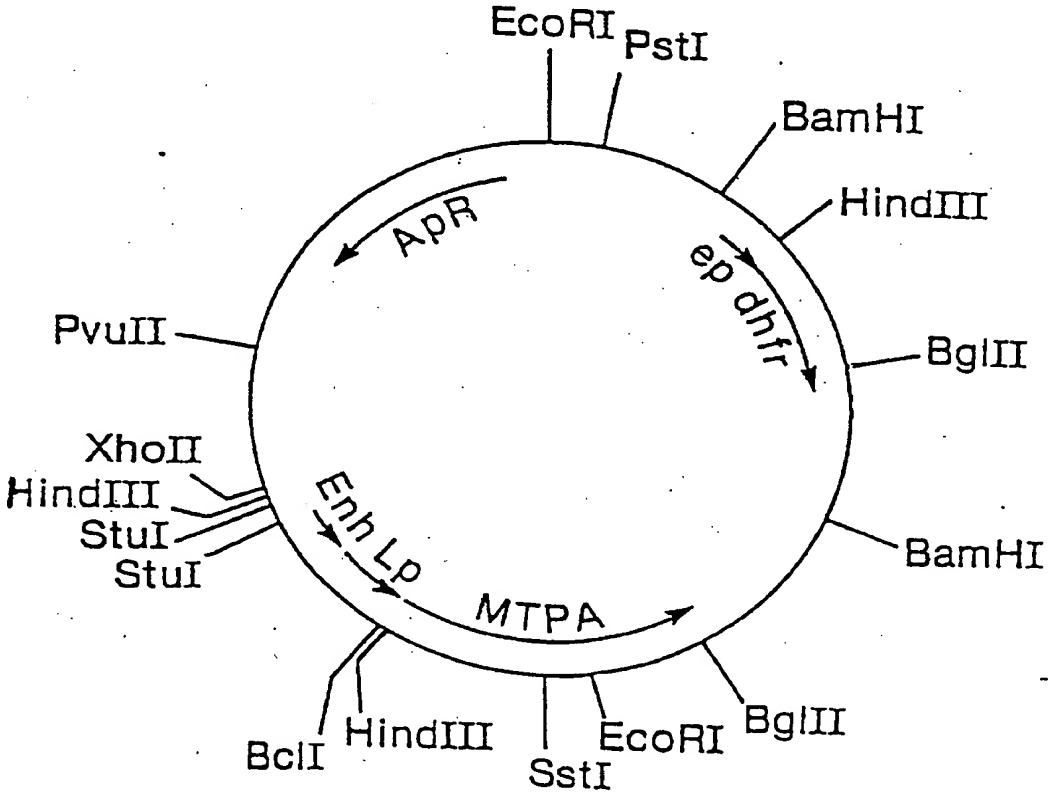

EcoRI
PstI
BamHI
HindIII
BglII
BamHI
BglII
EcoRI
SstI
HindIII
BclI
StuI
StuI
HindIII
XhoII
PvuII
ApR
ep dhfr
Enh Lp
MTPA

Figure 21

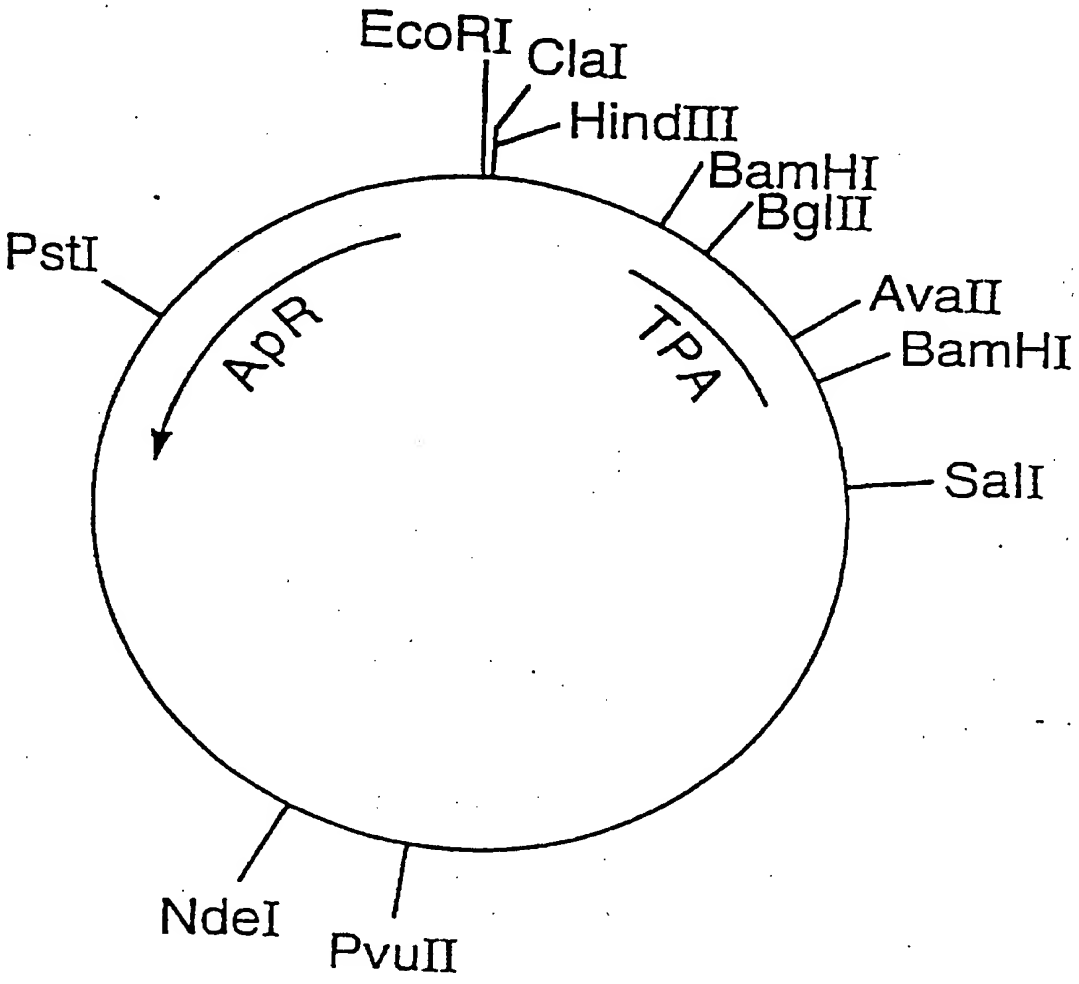

EcoRI
ClaI
HindIII
BamHI
BglII
AvaII
BamHI
SalI
PvuII
NdeI
PstI
ApR
TPA

Figure 22

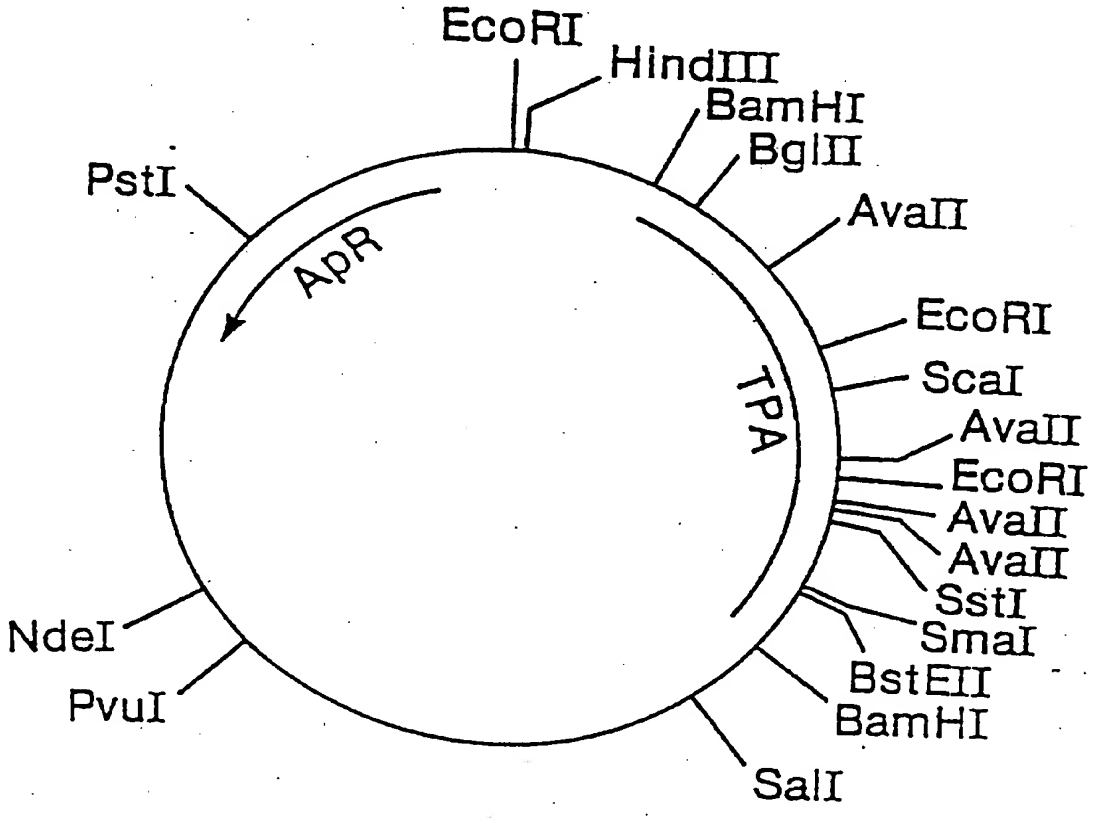

EcoRI
HindIII
BamHI
BglII
AvaII
EcoRI
ScaI
AvaII
EcoRI
AvaII
AvaII
SstI
SmaI
BstEII
BamHI
SalI
NdeI
PvuI
PstI
ApR
TPA

Figure 23

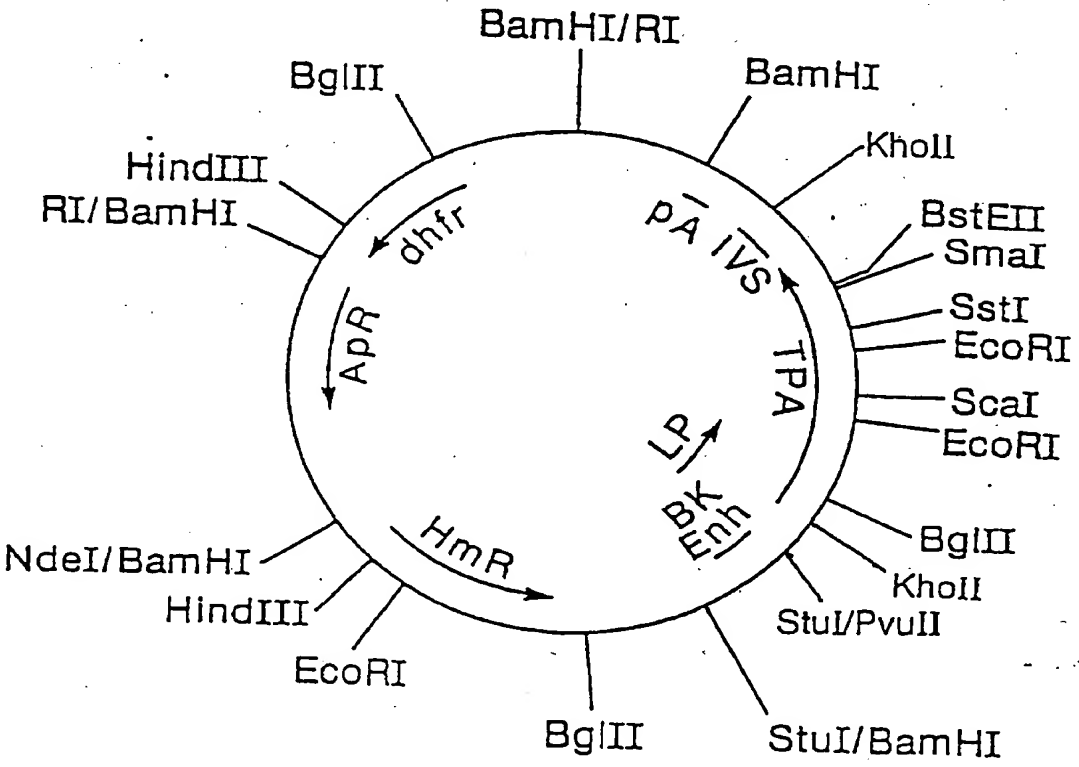
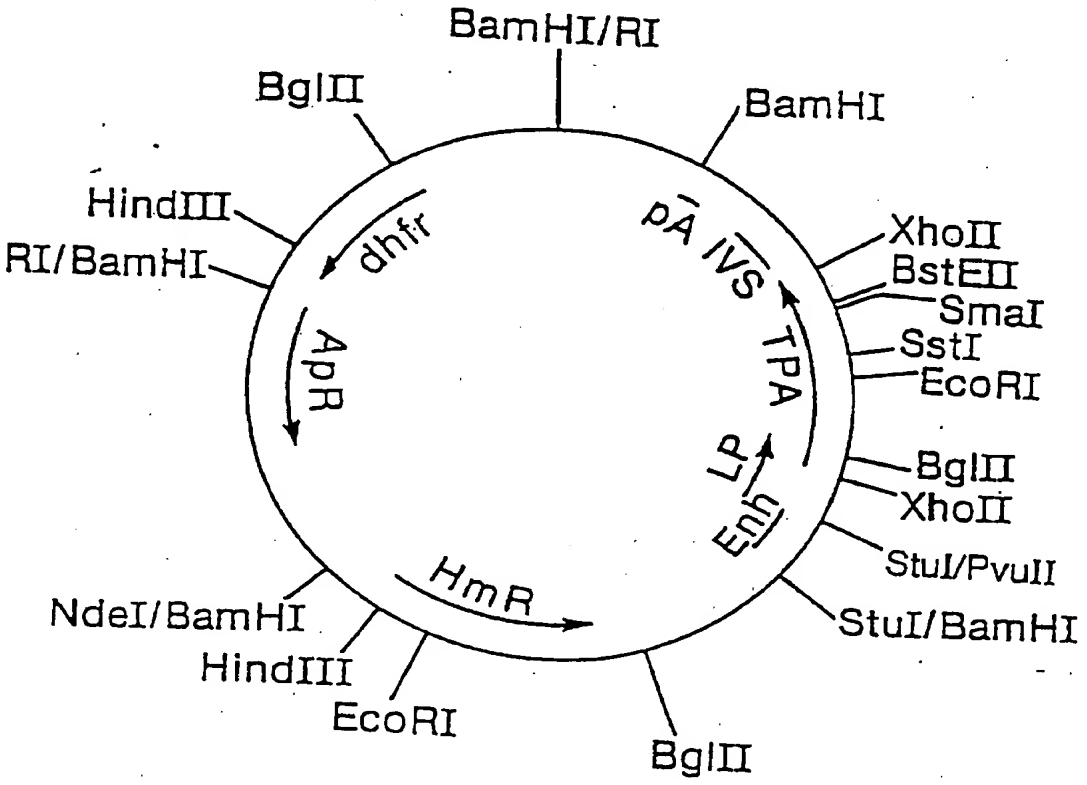

BamHI/RI
BglII
BamHI
KhoII
HindIII
RI/BamHI
BstEII
SmaI
SstI
EcoRI
ScaI
EcoRI
BglII
KhoII
StuI/PvuII
StuI/BamHI
BglII
EcoRI
HindIII
NdeI/BamHI
dhfr
ApR
pA
IVS
TPA
LP
BK Enh
HmR

Figure 24


BamHI/RI
BglII
BamHI
HindIII
RI/BamHI
dhfr
pA
IVS
XhoII
BstEII
SmaI
SstI
EcoRI
ApR
TPA
LP
Enh
BglII
XhoII
StuI/PvuII
StuI/BamHI
HmR
NdeI/BamHI
HindIII
EcoRI
BglII